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VOLUME XCV

BALTIMORE
THE JOHNS HOPKINS PRESS
1954

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July, 1954

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PUBLISHED MONTHLY
THE JOHNS HOPKINS PRESS, Baltimore-18, Maryland
Made in United States of America

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Subscription price. In the United States and its possessions $8.00 per year. In Canada $8.25 per year; in foreign countries $8.50 per year. Single numbers, $1.00.

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Entered as second class matter at the Baltimore, Md., Post Office. Acceptance for mailing at special rate of postage provided for in Section 1103, act of October 3, 1917. Authorized on July 3, 1918.

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ABSORPTION, EXCRETION AND METABOLIC FATE OF CHLORAL HYDRATE AND TRICHLOROETHANOL[1]

E. K. MARSHALL, JR. AND ALBERT H. OWENS, JR.[2]

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Received for publication April 22, 1954

Chloral hydrate was introduced as a therapeutic agent by Liebreich in 1869 (1). It was apparently widely used as a hypnotic and found quite satisfactory until the advent of a host of new synthetic hypnotics in the first two decades of the present century. These to a large extent replaced chloral hydrate without, however, any evidence of superiority. It is surprising that in the course of the eighty-five years since the introduciion of chloral hydrate so little knowledge of its pharmacology has accumulated.

The isolation of urochloralic acid (2) from the urine of patients receiving chloral hydrate, its identification as trichloroethanol glucuronide (3), Kulz's (4) demonstration of the narcotic activity of trichloroethanol and his finding that this substance also produced urochloralic acid were the main facts known about the metabolic fate of chloral hydrate prior to Butler's work in 1948 (5). Butler developed methods for the determination of trichloroethanol, chloral hydrate and trichloroacetic acid when present together in plasma. He found that when chloral hydrate is given intravenously to dogs, a small amount (3 to 4 per cent) is oxidized to trichloroacetic acid and a large amount, perhaps all of the remainder, is reduced to the alcohol. He also showed that the concentrations of trichloroethanol obtained are sufficient to account, in large part at least, for the narcotic effect of chloral hydrate.

In an individual taking a dose of chloral hydrate every evening for more than a month, it was found that the fraction of chloral hydrate being oxidized to trichloroacetic acid each day appeared to be of the order of 25 per cent instead of the 3 or 4 per cent found by Butler in the dog. This led to a study of the formation of trichloroacetic acid in several individuals receiving daily doses of chloral hydrate. When it was found that the amount of trichloroacetic acid formed varied from 5 to 47 per cent in different individuals, an investigation of certain aspects of the pharmacology of chloral hydrate and trichloroethanol[3] in man and dog was undertaken.

METHODS

Well trained female dogs were used for our studies. These were in the post-absorptive state. The drugs were given intravenously in isotonic saline solution and by mouth as an

[1] This investigation has been aided by a grant from the Life Insurance Medical Research Fund.

[2] E. R. Squibb Fellow in Pharmacology and Experimental Therapeutics.

[3] We wish to thank Mr. W. A. Lott of E. R. Squibb and Sons for supplying us with the trichloroethanol used in this investigation.

aqueous solution in 20 ml. of water per kilogram. Blood was obtained from a vein and urine by catheter. The blood was oxalated, centrifuged, and the plasma separated within one hour.

In man drugs were given by mouth usually before breakfast in solution in at least 100 ml. of water. Blood was drawn from an arm vein. Urine was preserved with a small amount of toluene.

Chloral hydrate and trichloroacetic acid were determined in plasma and urine by the Fujiwara reaction essentially as described by Butler (5). The sensitivity of the method was increased by using 3 ml. of the tungstate filtrate (3:25) and 2 ml. of 16–17 N sodium hydroxide. Trichloroethanol was determined by a new method, the basis of which is as follows. When a dilute solution of trichloroethanol is heated with dilute sodium hydroxide all the chlorine is removed as sodium chloride in the course of 2 to 3 minutes. Formaldehyde and carbon monoxide are found as the decomposition products.

When trichloroethanol is dissolved in sodium hydroxide and heated, an evolution of gas takes place, and reactions for an aldehyde are obtained. The gas was identified as carbon monoxide by reduction of the palladium ion to metallic palladium, and by formation of carbon monoxid-hemoglobin from oxyhemoglobin. The 2,4-dinitrophenylhydrazine derivative of the aldehyde present in solution after evolution of carbon monoxide was prepared and found to melt at 161–162° (the formaldehyde derivative melts at 164–165°). The dimedon derivative was prepared and found to melt at 185–187° when crude, but after recrystallization from aqueous ethanol at 190–192°. The dimedon derivative of formaldehyde was found to melt at 191°. A mixed melting point determination of the unknown and formaldehyde derivatives gave 191–192°.

The formaldehyde is determined by the method of Schryver (6) as modified by Matsukawa (7). The details of our procedure are as follows. Plasma proteins are precipitated with tungstic acid (8) usually in a 3:25 dilution. Urine may be diluted with tungstic acid or water. An aliquot of the tungstic acid filtrate (10 or 15 ml.) is placed in a flask, and trichloroethanol removed by distilling one-third to one-half of the volume into a 5 ml. volumetric flask placed in ice. Two ml. of the distillate are placed in a glass-stoppered tube, treated with 0.5 ml. of 0.35 N sodium hydroxide and heated for 3 minutes in a boiling water bath. The tube is then immersed in an ice-bath and 0.5 ml. of 0.35 N hydrochloric acid added. One ml. of a 0.1 M sodium citrate-hydrochloric acid buffer containing 7 per cent sodium chloride is then added.[4] This is followed by 1 ml. of chilled phenylhydrazine hydrochloride solution (freshly prepared, 0.5 per cent in 50 per cent alcohol). The tubes are placed in water at 40° for 12 minutes. Then 0.5 ml. potassium ferricyanide solution (2.5 per cent in 40 per cent alcohol) and 1 ml. of hydrochloric acid (concentrated acid diluted with equal volume of water) are added. The tubes are placed for 5 minutes in a water bath at 10–15°, and the color read in a Beckman spectrophotometer at 525 mu. A reagent blank is used for setting. The calibration curve is a straight line and is used from 0.01 to 1.0 mg. per cent trichloroethanol (in the distillate). Recoveries are complete within the error of the method when various concentrations of trichloroethanol are added to plasma or urine.

The trichloroethanol glucuronide is determined by the use of β-glucoronidase.[5] Urine is diluted with 0.15 M phosphate or acetate buffer (pH 6.0). Two ml. of diluted urine and 1 ml. of buffer are treated with 1 ml. of enzyme solution (400 and 200 units) and a drop of toluene, and incubated at 37.5° for 6 to 18 hours. This mixture is diluted to appropriate volume and the trichloroethanol removed by distillation. One ml. of plasma is treated with 2 ml. of the

[4] The sodium citrate-hydrochloric acid buffer should have a pH of 2.8 to 3.0 before adding the sodium chloride. To each 100 ml. of buffer is added 7 grams of sodium chloride.

[5] This was purchased from Sigma Chemical Company, St. Louis, Missouri.

buffer solution, and 1 ml. of enzyme solution (400 units). After addition of a drop of toluene, the mixture is incubated at 37.5°. Then the mixture is diluted to 15 ml. with tungstic acid solution, centrifuged, and an aliquot distilled. The fact that the supernatant liquid is frequently clouded may be disregarded.[6]

Using a sample of the sodium salt of trichloroethanol glucuronide,[7] the percentages hydrolyzed in different experiments were 96, 92, 109, 96, 96 and 97. Known amounts of this sodium trichloroethanol glucuronide were added to plasma and urine and completely recovered by the glucuronidase method.

Normal plasma and normal urine give a small blank. Using a 3:25 precipitation of proteins and collection of one-third volume as distillate, the plasma blank amounts to 0.1 mg. per cent or slightly less in human plasma and frequently less in dog plasma. This blank of plasma can be determined by extracting the trichloroethanol from the distillate with five volumes of benzene, and determining the residual "trichloroethanol". The urine blank appears to depend on the concentration of urine. We have subtracted the blank from all figures on plasma, but have not considered it in urine. Only a very slight error is involved.

By means of the methods which we have used, 0.1 mg. per cent of trichloroethanol, trichloroacetic acid, or chloral hydrate can be detected in plasma.

RESULTS

Oxidation of chloral hydrate during chronic administration to man

The percentage of orally administered chloral hydrate that is oxidized to trichloroacetic acid in man was determined as follows. Trichloroacetic acid when injected intravenously in man is apparently not destroyed and is excreted very slowly but quantitatively by the kidney (10). It follows that if an individual is receiving a constant dose of chloral hydrate and if its renal clearance is constant from day to day, equilibrium will be reached when the amount of trichloroacetic acid eliminated in the daily urine is exactly equal to the amount being produced in the body per day. Although we realize that these conditions are probably not fulfilled exactly, we have obtained from individuals taking a constant daily dose of chloral hydrate occasional 24 hour urines and samples of blood. These samples of plasma and urine were analyzed for trichloroacetic acid. When the concentration in plasma and/or the daily amount excreted became relatively constant, we have assumed equilibrium to have been attained.

In Table I are given the results of this part of our study. In this table are given data on 18 individuals given from 1 to 6 grams of chloral hydrate daily for various times. The concentrations of trichloroacetic acid in plasma at the end of our period of observation are given in Column 5. Renal clearance in liters of plasma cleared per day have been calculated and although not extremely accurate, do indicate the very slow excretion of trichloroacetic acid.

[6] The activity of β-glucuronidase preparation is checked by using different amounts when duplicates are done, and by standardization against phenolphthalein glucuronide.

[7] This specimen of the glucuronide was prepared by Butler (9). We wish to thank him for furnishing us with the preparation.

TABLE I

Oxidation of Chloral Hydrate to Trichloroacetic Acid in Man

NO.	WEIGHT KG	DOSE GM	DURATION DAYS	FINAL CONC. IN PLASMA MG. PER CENT	RENAL CLEARANCE LITERS PER DAY	PER CENT OXIDIZED
1	56	6.0	5	34	4.8	26
2	66	4.0	13	11	9.1	24
3	107	3.0	20	9	1.8	5
4	81	2.0	15	12	3.2	20
5	64	1.5	13	21	2.1	31
6	73	1.5	11	17	2.1	24
7	66	1.5	18	20	1.6	23
8	76	1.5	14	18	2.4	30
9	66	1.5	17	25	1.2	20
10	99	1.5	20	14	2.3	20
11	60	1.5	12	13	3.8	30
12	57	1.5	8	20	2.0	27
13	71	1.0	14	17	2.2	30
14	72	1.0	16	12	1.0	16
15	82	1.0	15	17	1.0	18
16	77	1.0	14	12	4.0	47
17	76	1.0	11	18	1.3	23
18	70	1.0	18	14	2.8	39

The quantity of a given daily dose of chloral hydrate oxidized after several days of drug administration is seen to vary from 5 to 47 per cent.[8] However, it must be realized that these are probably minimum values. As will be shown later, we have some evidence that the oxidation of chloral hydrate is greatest on the first days of administration and decreases with time.

To identify the substance giving the Fujiwara reaction as trichloroacetic acid the following experiment was performed. A 100 ml. sample of urine from a patient receiving chloral hydrate (4 grams per day) was acidified with 10 ml. of 12 N hydrochloric acid. The acidified urine was shaken with 100 ml. of ether, and then twice with 50 ml. portions of ether. The combined ether extracts were concentrated by evaporation at 60° under an air stream. Five ml. of an aqueous solution of 2-benzyl-2-thiopseudourea hydrochloride were added. The remaining ether was removed by evacuating the flask. The crystalline precipitate formed was collected by filtration and washed with water; 190 mg. equal to 99 mg. of trichloroacetic acid were obtained. The Fujiwara reaction showed in the 100 ml. of urine 98 mg. of trichloroacetic acid. The crude precipitate was recrystallized from water. It melted at 148–149° and when mixed with an authentic sample of the compound melted at 149–150°.

Analysis, calculated for $C_{10}H_{11}Cl_3N_2O_2S$: N, 8.50

Found: N, 8.78

Administration of chloral hydrate to the dog

The above findings in man are so different from those obtained by Butler (5) on intravenous injection of chloral hydrate in the dog, that a reinvestiga-

[8] Ahlmark and Forssman (11) state that when chloral hydrate is given orally to man, about one-half of the drug is excreted as trichloroacetic acid. No further details are given.

TABLE II

Concentration in Plasma, Renal Excretion and Apparent Distribution of Trichloroacetic Acid in the Dog Following the Intravenous Administration of 50 mg. per kg.

TIME HOURS	PLASMA MG PER 100 ML	URINE MG PER DAY	APPARENT DISTRIBUTION PER CENT BODY WEIGHT
24	10	138	39
48	6	113	50
72	4	91	57
96	3	51	62
120	2	29	81

tion of the behavior of the drug in the dog was undertaken. The discrepancy between Butler's results obtained in the dog and our results in man was found to be due to three factors; namely, the method of administration of chloral hydrate, the slow production of trichloroacetic acid after the first few hours, and a species difference in the behavior of trichloroacetic acid in the dog and man.

The species difference is easily shown by a repetition in the dog of the experiments of Paykoç and Powell (10) in man. A typical experiment was as follows.

Dog, Tillie, 12.5 kg. was given intravenously 50 mg. per kilogram of trichloroacetic acid as the sodium salt. The amounts of trichloroacetic acid in plasma and urine were determined daily. From this data the apparent volume of distribution of the acid was calculated. Table II gives the results.

Since there is a marked increase in the apparent "trichloroacetic acid space" as calculated from day to day, it is apparent that either the acid is excreted to a large extent by some other route than the kidney or that it is destroyed in the organism of the dog. The second possibility is probably the correct one.

When chloral hydrate is given by mouth to the dog, absorption appears to be extremely rapid, trichloroethanol and trichloroacetic acid appearing in the plasma. Typical experiments are shown in Figure 1. In the experiment illustrated in Figure 1A, more than 26 per cent of the administered chloral hydrate was oxidized.[9]

Administration of trichloroethanol to the dog

When trichloroethanol is given by mouth to the dog it is rapidly and completely absorbed. This is deduced from observing that the peak concentration in the plasma is reached in 15 or 30 minutes and that just as much material is excreted in the urine in 2 hours from oral administration as from intravenous.

[9] This is calculated by assuming the "trichloroacetic acid space" of the dog to be 20 per cent of body weight (5). The dog weighed 17 kilograms, and had 69 mg. per liter of trichloroacetic acid in the plasma at 24 hours.

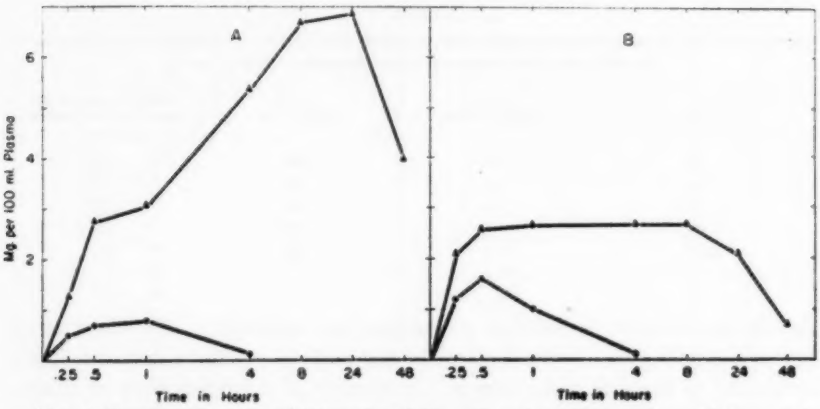

FIG. 1. Concentration of trichloroethanol ● — ● and trichloroacetic acid ▲ — ▲ in plasma following the oral administration of chloral hydrate, 60 mg. per kg. A and B compare experiments in dogs, Liz and Gretchen.

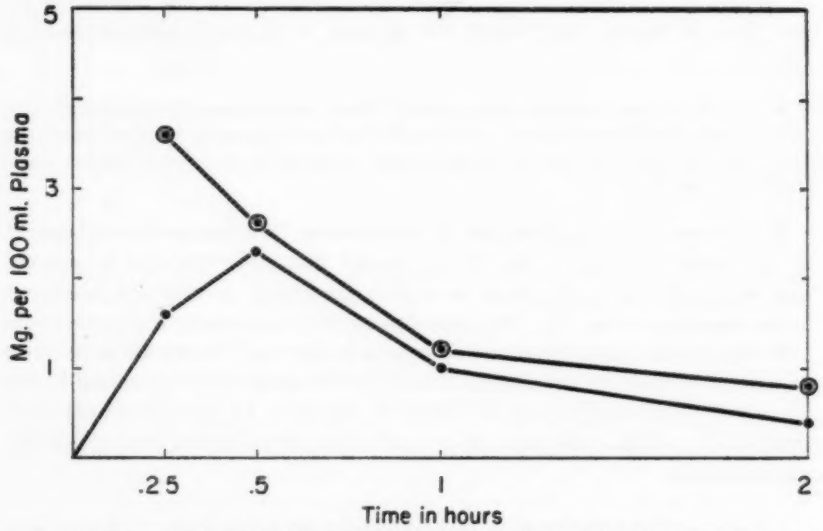

FIG. 2. Concentration of trichloroethanol in plasma following the administration of trichloroethanol, 54 mg. per kg., intravenously ⊙ — ⊙ and orally ● — ●. The renal excretion of total trichloroethanol was identical (275 and 276 mg.) during both 2 hour periods. (Dog, Theda).

A typical plasma concentration—time curve after oral and after intravenous administration of trichloroethanol is shown in Figure 2.

We have made a few observations on the plasma binding of both trichloroethanol and trichloroacetic acid, utilizing the method of Davis (12). Recon-

TABLE III

Plasma Binding of Trichloroethanol and Trichloroacetic Acid

TRICHLOROETHANOL		TRICHLOROACETIC ACID	
Concentration in plasma*	Per cent bound	Concentration in plasma*	Per cent bound
0.9	35	2.6	88
2.0	41	4.1	87
4.0	38	7.3	83
7.7	39	11.1	72
		22.5	71

* Mg. bound and unbound drug per 100 ml. plasma.

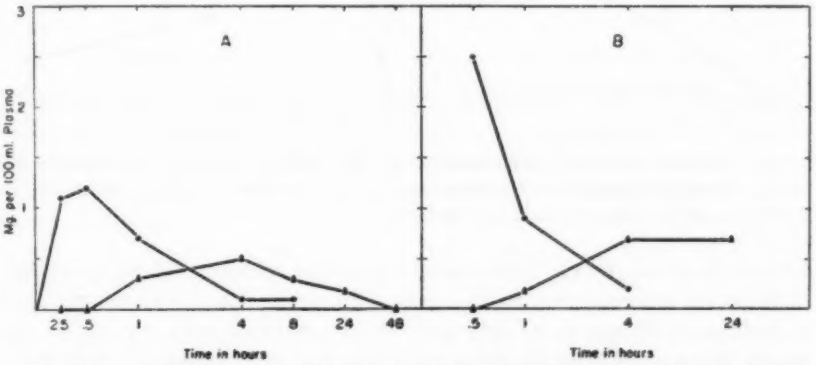

FIG. 3. Concentrations of free trichloroethanol ● — ●, and trichloroacetic acid ▲ — ▲ in plasma following the administration of trichloroethanol, 54 mg. per kg., orally (A) and intravenously (B) to dog, Gretchen.

stituted lyophilized human plasma[10] was used. Results are given in Table III. The high binding of trichloroacetic acid does not seem sufficient to account for its slow renal excretion; probably, considerable reabsorption occurs in the renal tubule. Furthermore, it seems unlikely that trichloroacetic acid is distributed in the extracellular compartment alone.

In several experiments designed to study the absorption and excretion of trichloroethanol in the dog our results appeared to completely confirm the conclusion of Butler that trichloroacetic acid was not formed from trichloroethanol. However, when it was subsequently found that trichloroacetic acid is produced from trichloroethanol in man, a reexamination of the behavior of this substance in the dog indicated that as in man small amounts of trichloroacetic acid were indeed formed. In the first hour or so after administration of trichloroethanol none or only traces of trichloroacetic acid can be detected in plasma, but later definite amounts are present. Figure 3 gives representative

[10] We wish to thank Dr. L. Earle Arnow of Sharp and Dohme Inc. for supplying this material.

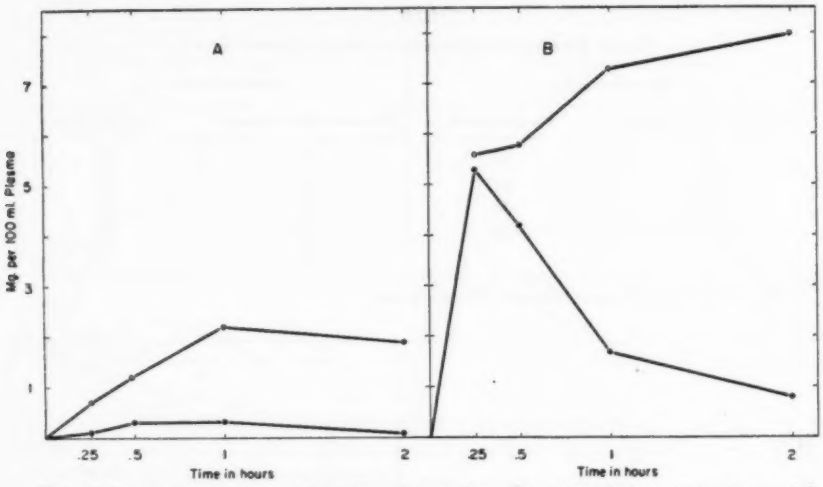

FIG. 4. Concentrations of trichloroethanol, free ● — ● and total ○ — ○ in plasma following the oral administration of trichloroethanol. In A the dose was 27 mg. per kg. and in B 108 mg. per kg. (Dog, Tana in both instances.)

data on the production of trichloroacetic acid from trichloroethanol in the dog. It might be supposed that trichloroethanol is oxidized to trichloroacetic acid in amounts sufficient to be detectable by our methods only when given by mouth. However, Figure 3B shows the production of trichloroacetic acid when the trichloroethanol is given intravenously.

A large amount of the trichloroethanol in the plasma of the dog is present as trichloroethanol glucuronide. The concentrations of total and free trichloroethanol in plasma from a relatively small and large dose are shown in Figure 4.

The proportion of chloral hydrate or trichloroethanol that has been reported to be excreted in the urine of dogs and rabbits as trichloroethanol and trichloroethanol glucuronide is in the range of 40 to 60 per cent (9). Our data indicate that only a small amount of free trichloroethanol is present in urine. In determinations of free and total trichloroethanol in 17 samples of urine of dogs, the average percentage of free trichloroethanol was 0.83 (range 0.02 to 2.8). By using the more accurate β-glucuronidase method we have accounted for much more of the trichloroethanol as glucuronide in the dog. Our data on excretion in 2 dogs are given in Table IV. The first figure under each dose is that obtained by intravenous injection, the second by oral administration. If one considers that a certain amount of trichloroethanol is oxidized to trichloroacetic acid in the dog, it is clear that most, if not all, of the drug, which has been administered, is accounted for.

TABLE IV

*Renal Excretion of Total Trichloroethanol in Dogs**

TIME IN HOURS	BROWNHEAD							THEDA						
	Dose, mg per kg							Dose, mg per kg						
	13.5	27		54		108		13.5	27		54		108	
	ST	IV	ST	IV	ST	IV	ST	ST	IV	ST	IV	ST	IV	ST
2	42	33	24	31	38	16	21	43	35	52	34	36	31	30
8	71	52	53	68	73	63	60	81	69	77	80	80	70	71
24	84	58	61	74	83	78	72	88	78	84	88	91	78	82

* Expressed as a cumulative percentage of the dose which was administered intravenously (IV) or by stomach tube (ST).

Administration of chloral hydrate and trichloroethanol to man

The behavior of chloral hydrate when administered orally to man is very similar to its behavior in the dog. The substance is apparently very rapidly absorbed and relatively high concentrations of trichloroacetic acid are found in plasma coincident with the appearance of trichloroethanol. Figure 5 shows representative data on the metabolic fate of chloral hydrate. Additional data are given in Table V. As can be seen from these data much larger amounts of trichloroacetic acid are formed in man than in the dog, and the early-rapid and slow-late formation of this substance is more evident in man.

When trichloroethanol is given to man, absorption appears to be extremely rapid, and although trichloroacetic acid is formed from the alcohol, none or only traces of this substance can be detected in the plasma in the first hour or two after administration of the drug. In contrast to the dog, only small amounts

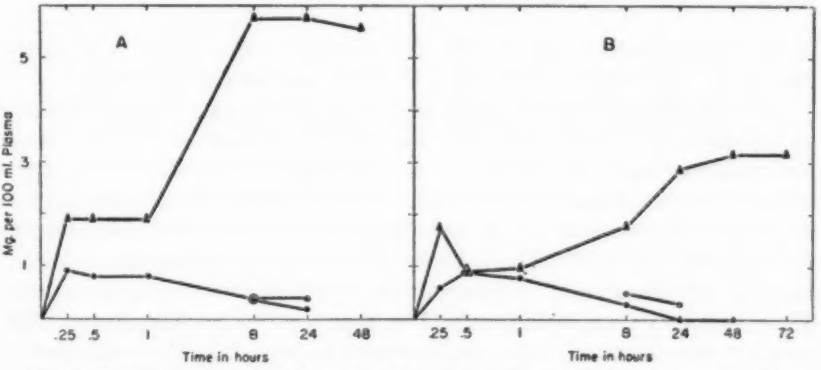

FIG. 5. Concentrations of trichloroethanol, free ● — ● and total ○ — ○ and trichloroacetic acid ▲ — ▲ in plasma following the oral administration of chloral hydrate, 16.5 mg. per kg. to man. A and B compare experiments in Wa and Bo.

TABLE V

Concentrations of Trichloroethanol and Trichloroacetic Acid in Plasma of Man Following the Oral Administration of Chloral Hydrate, 16.5 mg per kg.

TIME IN HOURS	P		Mc		Bo		We		Fu		Wa	
	TCE*	TCA†	TCE	TCA	TCE	TCA	TCE	TCA	TCE	TCA	TCE	TCA
.25	0.4/0.4	0.2	1.3/1.3	1.6	1.8	0.6	0.3	0.3	1.3	1.4	0.9	1.9
.5					0.9	0.9	0.7	1.3	0.8	1.4	0.8	1.9
1					0.8	1.0	0.8	1.5	0.7	1.4	0.8	1.9
2	0.7/0.8	1.9	0.7/0.8	1.6								
8					0.3/0.5	1.8	0.4/0.2	4.3	0.2/0.3	1.9	0.4/0.3	5.8
24					0.0/0.3	2.9	0.1/0.2	5.4	0.0/0.3	2.5	0.2/0.3	5.8
48					0.0	3.2		5.2		2.7		5.6
72						3.2						

* Mg per 100 ml plasma of free trichloroethanol or FREE/TOTAL as indicated.

† Mg per 100 ml plasma of trichloroacetic acid.

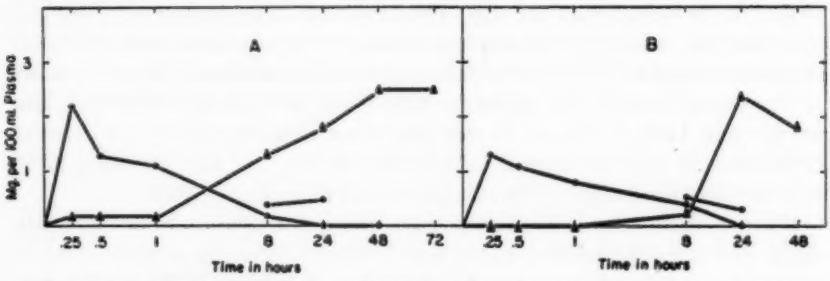

FIG. 6. Concentrations of trichloroethanol, free ● — ● and total ○ — ○ and trichloroacetic acid ▲ — ▲ in plasma following the oral administration of trichloroethanol, 15 mg. per kg. to man. A and B compare experiments in Wo and Fi.

of the trichloroethanol are present in conjugated form. Figure 6 gives the data of two experiments and in Table VI additional data are presented.

In Tables VII and VIII are given data on the percentages of chloral hydrate and trichloroethanol recovered from the urine in 24 hours as trichloroethanol and trichloroethanol glucuronide. In determinations of the free and total trichloroethanol in 15 samples of the urine after administration of either chloral hydrate or trichloroethanol, the average percentage of free trichloroethanol was 4.6 (range 0.5 to 19). This is higher than that found in the dog. It is seen that the amount of total trichloroethanol recovered is variable, from 16 to 35 per cent for chloral hydrate and 17 to 40 per cent for trichloroethanol. The data also show a marked difference between dog and man in the metabolism of trichloroethanol; in the dog a large amount, up to 40 per cent or more, may be excreted in the first 2 hours as glucuronide and a large part of the remainder in the next 6 hours; whereas, in man, less than 10 per cent is eliminated in the first 2 hours and no more than 25 per cent in the next 6 hours.

TABLE VI

Concentrations of Trichloroethanol and Trichloroacetic Acid in Plasma of Man Following the Oral Administration of Trichloroethanol, 15 mg per kg.

TIME IN HOURS	M		O		Ri		S		Wo		Fi	
	TCE*	TCA	TCE	TCA	TCE	TCA†	TCE	TCA	TCE	TCA	TCE	TCA
.25	1.3/1.9		0.9/0.9		1.3/1.3	0.0	0.6/0.6	0.0	2.2	0.2	1.3	0.0
.5					0.9/0.9	0.0	1.5/1.4	0.0	1.3	0.2	1.1	0.0
1			1.4/1.3		0.8/0.8	0.0	0.7/0.7	0.0	1.1	0.2	0.8	0.0
2	0.5/0.9		0.6/0.8									
8									0.2/0.4	1.3	0.4/0.5	0.3
24									0.0/0.5	1.8	0.0/0.3	2.4
48									0	2.5		1.8
72										2.5		

* Mg per 100 ml. plasma of free trichloroethanol or FREE/TOTAL as indicated.
† Mg per 100 ml plasma of trichloroacetic acid.

TABLE VII

*Renal Excretion of Chloral Hydrate as Total Trichloroethanol in Man**

TIME IN HOURS	P	Mc	Bo	Fu	We	Wa
2	5	5	4	7	3	7
8	16	14	18	23	9	17
24	31	26	31	35	16	26

* Expressed as a cumulative percentage of the administered dose of chloral hydrate, 16.5 mg per kg.

TABLE VIII

*Renal Excretion of Total Trichloroethanol in Man**

TIME IN HOURS	M	O	Ro	K	Bl	O	Ri	S	Wo	Fi
2	7	9	5	7	4	6		8	8	5
8	15	18				14	9	23	20	19
24	26	27	18	28	23	25	17	40	36	38

* Expressed as a cumulative percentage of the administered dose of trichloroethanol, 15 mg per kg.

In spite of the relatively small excretion of glucuronide in the urine of man after administration of chloral hydrate or trichloroethanol, a large part of the dose of the drugs can be accounted for by considering the trichloroacetic acid formed. Thus, in the case of *We* (Table V and VII) given 1 gram of chloral hydrate (equivalent to 900 mg. trichloroethanol) 140 mg. were excreted in the urine as free and conjugated trichloroethanol. The concentration of trichloroacetic acid in plasma was 54 mg. per liter at 24 hours. The subject weighed 61 kg. On the assumption that the trichloroacetic acid had an apparent volume of distribution of 25 per cent of the body weight (10), we can calculate that 820 mg. of trichloroacetic acid (equal to 738 mg. of trichloroethanol) were

present in the organism. At 24 hours, the concentration of total trichloroethanol in plasma was 2 mg. per liter, which assuming it distributed in total body water, would give 81 mg. in the body. This gives a recovery of 106 per cent of the dose administered. Using the same method of calculation, we find with subjects *Wa*, *Bo* and *Fu* given chloral hydrate, recoveries of 125, 89 and 84 per cent respectively. In subject *Wo* (Tables VI and VIII) weighing 87 kg. and given 1300 mg. of trichloroethanol, 472 mg. of total trichloroethanol were eliminated in the urine in 24 hours. The trichloroacetic acid concentration in the plasma was 18 mg. per liter. On the basis of distribution in one-fourth of the body weight, the individual contained 392 mg. of trichloroacetic acid corresponding to 354 mg. of trichloroethanol. At 24 hours the concentration of trichloroethanol glucuronide in the plasma was 5 mg. per liter, which assuming it distributed in total body water, would mean 290 mg. of trichloroethanol in the body. This gives a total recovery of 86 per cent. Similar calculation on Fi (Tables VI and VIII) gives a recovery of 87 per cent. When one considers that the "trichloroacetic acid space" is variable in different individuals, and that small amounts of trichloroacetic acid are excreted in the first 24 hours, the percentage recoveries calculated above would appear to indicate that most, if not all, of the chloral hydrate and trichloroethanol is accounted for by total trichloroethanol excretion and formation of trichloroacetic acid.

Assuming that trichloroacetic acid is distributed in 25 per cent of the body weight, we can calculate the amount of this substance formed from single doses of chloral hydrate and trichloroethanol. Taking the maximum concentration of trichloroacetic acid found in plasma, and neglecting the small amount excreted in the urine, we have 40, 49, 81 and 87 per cent oxidation with chloral hydrate, and 35 and 38 per cent with trichloroethanol. A similar calculation shows that at one hour after the dose, 15, 21, 23 and 29 per cent of chloral hydrate has been oxidized. In contrast in three instances there was in one hour no detectable oxidation of trichloroethanol; only 3 per cent was oxidized in another individual.

Chloral hydrate in blood

Experiments have been performed in an attempt to determine the amount of chloral hydrate present in plasma at various times after its ingestion in both dog and man. In experiments already mentioned in regard to the absorption, excretion and metabolic fate of chloral hydrate in both dog and man, no chloral hydrate could be detected in any of the samples of plasma. However, relatively small doses were given, and the first sample of blood was not drawn until 15 minutes after ingestion of the drug. In the dog, when large doses are given or when blood is taken early in an experiment with moderate doses, some chloral hydrate can be demonstrated to be present in plasma. An ex-

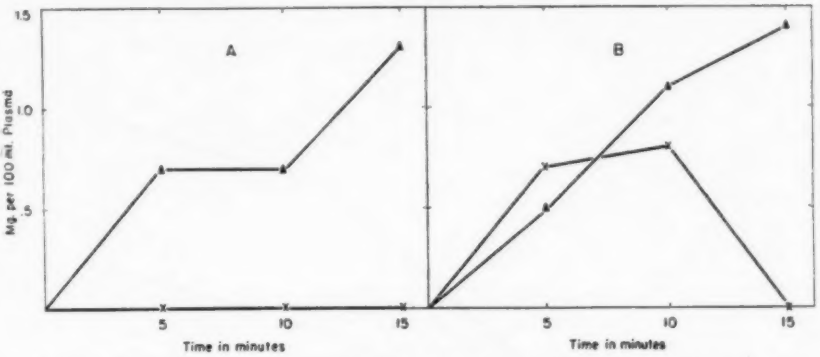

FIG. 7. Concentrations of chloral hydrate × — × and trichloroacetic acid ▲ — ▲ in plasma following the oral administration of chloral hydrate. In A the dose was 15 mg. per kg. and in B, 60 mg. per kg. (Dog, Brownhead, in both instances).

amination of Figure 7 shows that when 60 mg. per kg. were given to a dog, chloral hydrate was present in samples of blood taken at 5 and 10 minutes but not in a sample at 15 minutes. When 15 mg. per kg. were given to the same dog, no chloral hydrate could be detected in blood at either 5, 10 or 15 minutes after ingestion of the drug. Another factor conditioning the amount of chloral hydrate to be found in plasma is the rapidity of absorption from the intestine, presumably the rate of passage of the drug through the liver. This is evident from the data given in Figure 8.

Administration of 16.5 mg. per kg. of chloral hydrate to man in the post-

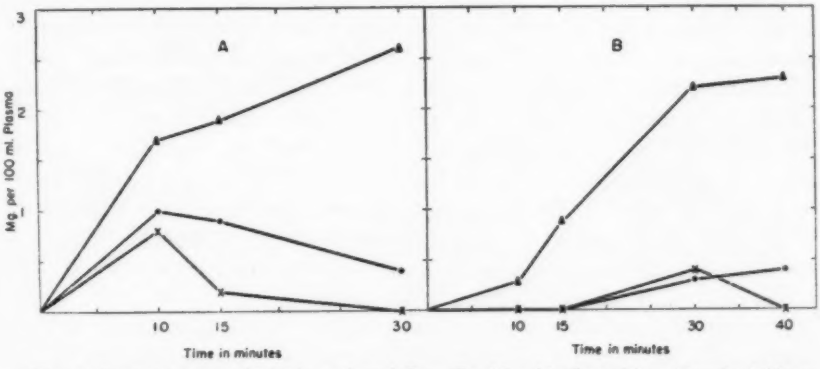

FIG. 8. Concentrations of trichloroethanol ● — ●, chloral hydrate × — × and trichloroacetic acid ▲ — ▲ in plasma following the oral administration of chloral hydrate, 53 mg. per kg., in aqueous solution (A) and 58 mg. per kg. in thick walled gelatin capsules (B). (Dog, Liz, in both instances).

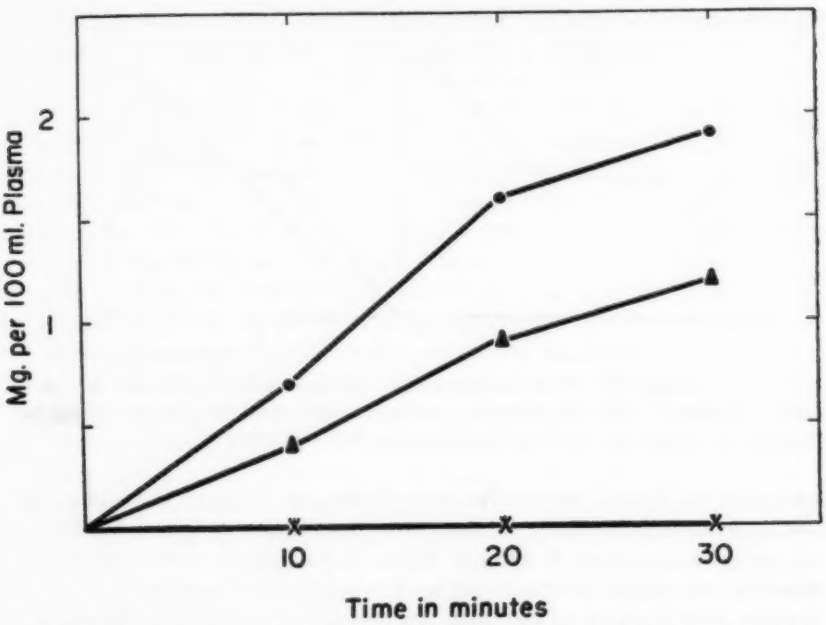

FIG. 9. Concentrations of trichloroethanol ● — ●, chloral hydrate × — × and trichloroacetic acid ▲ — ▲ following the oral administration of chloral hydrate, 30 mg. per kg. to man (Ar).

absorptive state has resulted in the detection of no chloral hydrate in samples of blood drawn 15, 30 and 60 minutes after ingestion of drug. In 2 individuals, 15 and 30 mg. per kg. respectively of chloral hydrate in solution were given at the time when it would most commonly be administered as a hypnotic (about 3 hours after the evening meal). Samples of blood taken at 5, 10 and 15 minutes showed no detectable chloral hydrate. The experiment was repeated on 2 additional individuals and samples of blood obtained 10, 20 and 30 minutes after the administration of the drug. In none of these samples could chloral hydrate be detected.[11] The data on one of these individuals is given in Figure 9. Absorption as judged by the concentrations of trichloroethanol and trichloroacetic acid present in plasma appears to be somewhat slower in these experiments than when the drug is given before breakfast.

Although the above experiments demonstrate that in the doses used for hypnosis, chloral hydrate cannot contribute to the narcotic effect, it is still

[11] The samples of blood obtained in these experiments were immediately placed in chilled centrifuge tubes and kept in an ice-bath until centrifuged. This was done within a period not exceeding 40 minutes.

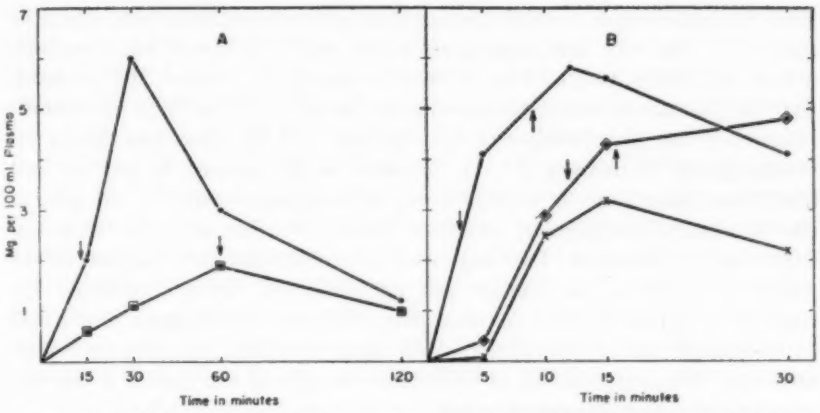

FIG. 10. A. Concentrations of trichloroethanol in plasma following the oral administration of trichloroethanol, 90.8 mg. per kg. in gelatin capsules ● — ● and chloral hydrate, 100 mg. per kg. in gelatin capsules ⊡ — ⊡. The arrow (↓) indicates the appearance of ataxia. (Dog, Fannie, in both instances).

B. Concentrations of trichloroethanol ● — ● in plasma following the oral administration of trichloroethanol, 108 mg. per kg., and of trichloroethanol ◈ — ◈ and chloral hydrate × — × following the oral administration of chloral hydrate, 120 mg. per kg. The first arrow (↓) indicates the appearance of ataxia and the second arrow (↑) the time at which the dog could no longer stand. In another similar pair of experiments ataxia appeared at trichloroethanol concentrations of 2.7 mg. per 100 ml. plasma following the oral administration of both trichloroethanol and chloral hydrate. (Dog, Theda, in all instances.)

possible that chloral hydrate when present in blood may have some depressant effect upon the central nervous system. Some evidence on this question was obtained in experiments where relatively large doses of chloral hydrate and trichloroethanol were given alternately to the same dogs. In one experiment (Figure 10A) no attempt was made to determine chloral hydrate in the plasma. It can be seen, however, that the concentrations of trichloroethanol in the plasma are identical when symptoms appear whether trichloroethanol or chloral hydrate was given. In another experiment (Figure 10B) of the same type, chloral hydrate was determined in the plasma. It can be seen that the symptoms correspond to the concentrations of trichloroethanol in plasma. The presence of equal concentrations of chloral hydrate and trichloroethanol gives an identical pharmacological effect as that given by the same concentration of trichloroethanol alone.

The above data suggest strongly that trichloroethanol is the only active constituent responsible for depression of the central nervous system when either chloral hydrate or trichloroethanol is administered. However, two other substances present in plasma must be considered; namely, trichloroethanol glucuronide and trichloroacetic acid. Kulz (4) reported that the glucuronide

given in large dosage, to rabbit, dog or man had no hypnotic action. Later, he found (13) that very large doses given to the rabbit by mouth had a narcotic action, but that it took an hour or more for the effect to occur. It is probable that the glucuronide was decomposed either by the colon bacilli in the rabbit's intestine or the glucuronidase of the organism, and the effect was due to trichloroethanol. Hermann's (14, 15, 16) work on the absence of any narcotic effect from large doses of trichloroacetic acid appears conclusive, in spite of the subsequent contradictory report of Mayer (17). Our own experience confirms that of Hermann. Two dogs were given 300 mg. per kilogram of trichloroacetic acid as the sodium salt intravenously. Neither exhibited the slightest symptom. A third dog was given 1000 mg. per kilogram of the acid as its sodium salt intravenously. Aside from vomiting, no symptoms were observed. The concentration of trichloroacetic acid in the plasma 5 minutes after injection was 300 mg. per cent.

DISCUSSION

Butler concluded that when chloral hydrate was injected into dogs the quantities of free trichloroethanol formed were sufficient to account, in large part at least, for the narcotic effect. The results of the present investigation prove conclusively that, when chloral hydrate is given to man in therapeutic doses, the hypnotic effect is due entirely to trichloroethanol. This conclusion follows from the facts that no chloral hydrate (or a small undetectable trace) is present in plasma after therapeutic doses of the drug, and that trichloroethanol is the only substance present in plasma having narcotic properties. Our results do not prove conclusively that chloral hydrate *per se* is entirely devoid of narcotic properties; but they do show that, if chloral hydrate is a narcotic, it is one of a very low order of activity compared to trichloroethanol. This follows from our finding that not only are the symptoms of central nervous system depression of the same magnitude when the concentrations of trichloroethanol in plasma are the same whether arising from the administration of large doses of trichloroethanol or chloral hydrate, but that the presence of the same concentration of chloral hydrate as of trichloroethanol does not intensify the depression over that given by trichloroethanol alone.

When given day by day in a constant dose it appears that 5 to 47 per cent of chloral hydrate may be oxidized each day. However, if one examines the production of trichloroacetic acid after a single dose, oxidation appears to be in some cases as much as 87 per cent. Possibly, the amount of trichloroacetic acid formed from chloral hydrate decreases with time as a constant daily dose is given.

Further investigation of the production of trichloroacetic acid from chloral hydrate shows that it occurs in two apparently separate stages. Coincident with the absorption of chloral hydrate from the intestine, trichloroacetic acid

appears in the plasma in concentrations which do not change appreciably for some time; later a very slow production of trichloroacetic acid, which may last for 48 hours, is evident. The first rapid phase in the production of trichloroacetic acid is seen with chloral hydrate and not with trichloroethanol; the second slower production occurs from trichloroethanol.

Trichloroethanol is absorbed from the gastrointestinal tract extremely rapidly in both dog and man when in the post-absorptive stage. The concentrations of free trichloroethanol in plasma are much higher and persist longer in man than in the dog due mainly to the fact that the dog conjugates the material more quickly and to a greater extent than man. Since the dog apparently destroys trichloroacetic acid and man does not, it is difficult to compare the relative amounts of this substance produced in the two species. But much less must be found in the dog as he excretes more than twice as much trichloroethanol as man in the urine as glucuronide.

It appears that the whole narcotic activity of chloral hydrate is due to the trichloroethanol formed by reduction. Since during the process of absorption, a certain amount of chloral hydrate is converted to the inactive trichloroacetic acid rather than reduced to the active trichloroethanol, it would seem that trichloroethanol should be a more potent and consistent hypnotic than chloral hydrate.

SUMMARY

When administered to dog or man, both chloral hydrate and trichloroethanol are absorbed rapidly and completely. Most, if not all, of each drug can be accounted for as trichloroethanol, trichloroethanol glucuronide and trichloroacetic acid.

Trichloroacetic acid is formed from both chloral hydrate and trichloroethanol. The production of trichloroacetic acid from chloral hydrate starts during absorption of the drug and is completed within the first hour. However, the trichloroacetic acid formed from trichloroethanol appears more slowly and may continue to accumulate for 24 to 48 hours.

In man, the depressant effect of chloral hydrate when given in therapeutic doses appears to be due entirely to trichloroethanol; no chloral hydrate can be detected in the blood. In dogs, receiving much larger doses, some chloral hydrate can be detected in blood. However, if chloral hydrate is a depressant *per se*, its potency is of a much lower order than that of trichloroethanol.

A comparison of their pharmacological characteristics suggests that trichloroethanol should be a more potent and more reliable hypnotic than chloral hydrate.

ACKNOWLEDGMENTS

We wish to thank Dr. Emmett L. Buhle for assistance in identification of the degradation products of trichloroethanol as carbon monoxide and formaldehyde and of trichloroacetic acid in the urine

of an individual receiving chloral hydrate. We wish also to thank Sara Ann Verplanck for technical assistance throughout the entire investigation.

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CONSIDERATIONS CONCERNING THE PATHWAYS OF SYNTHESES IN LIVING MATTER*

Synthesis of Glycogen from Non-Carbohydrate Precursors

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Received for publication May 3, 1954

INTRODUCTION

Syntheses are often taken to be a reversal of break-down reactions. Whilst this is true in respect to the over-all effect, it is not necessarily correct in respect to enzymic mechanisms and metabolic pathways. In a complex sequence of stages some individual steps may be identical in break-down and in synthesis, but this never does apply to all steps. To elaborate this thesis is the main object of this lecture.

I must first define the manner in which I shall use the term 'synthesis'. The term is sometimes used with reference to the preparation of a substance from other chemicals. For the purpose of this paper I arbitrarily define synthesis as a reaction in which larger molecules are built up from smaller ones *with loss of free energy*. The endergonic nature of the reaction is, in my context, an essential feature of a synthesis.

Needless to say syntheses are endergonic processes only when looked upon in isolation. Examples are the synthesis of carbohydrate from lactate, of glycogen from glucose, or of fatty acids from acetic acid. It follows from the principles of thermodynamics that these reactions must be coupled with exergonic processes in such a way that the over-all balance of all processes is no longer endergonic. One of the main problems in the study of syntheses is the nature of the coupling with exergonic processes.

I would like to consider the principles that are involved in a study of biochemical syntheses by discussing some recent work on the synthesis of carbohydrate from non-carbohydrate precursors, especially from lactate or pyruvate.

Thermodynamic aspects of carbohydrate synthesis. Whilst it is certain from experiments with isotopic compounds and also from earlier work on diabetic and on phloridzin-poisoned animals, that three-carbon compounds such as lactate and pyruvate may be converted into carbohydrate in animal tissues and other living cells, the pathway of this synthesis is by no means fully understood. It is true that all steps of the anaerobic lactic acid formation from

* Lecture I of The Herter Lectures delivered at The Johns Hopkins Hospital February 24, 1954.

carbohydrate have been shown to be reversible, and the possibility of a synthesis of glucose from lactate or pyruvate by reversal of the fermentation therefore exists. Most authors assume (see e.g. Topper & Hastings, (1); Fruton & Simmonds, (2) that carbohydrate is in fact formed by the same route by which it is broken down. There are however difficulties from the quantitative point of view when the thermodynamic properties of the system are considered, the thermodynamic equilibrium of carbohydrate fermentation being very unfavourable for a reversal. For thermodynamic consideration the changes associated with the fermentation of a glucose equivalent of glycogen may be formulated as follows*:

(1) $$\text{glycogen} \rightarrow 2\ \text{lactate}^- + 2H^+ \quad (\Delta G = -56.7\ \text{Kcal})$$

(2) $$3[ADP^{3-} + HPO_4^{2-} + H^+ \rightarrow ATP^{4-} + H_2O] \quad (\Delta G = +3 \times 11.3\ \text{Kcal})$$

The sum of these two reactions is

(3) $$\text{glycogen} + 3\ ADP^{3-} + 3\ HPO_4^{2-} + 3H^+ \rightarrow 2\ \text{lactate}^- + 3\ ATP^{4-} + 3\ H_2O \quad (\Delta G = -22.8\ \text{Kcal})$$

It follows from this ΔG value that appreciable quantities of glycogen can only be formed if the concentration of ATP is extremely high in relation to those of ADP and inorganic phosphate. The ratio of the concentrations of ATP/ADP must be of the order of 10^{16}. Observed figures for this ratio are of the order of 10 or less and in view of the high activity of ATPases in most tissues it is difficult to visualise ratios of the postulated order, even if special spatial arrangements exist which separate reactants. Thus an appreciable synthesis of glycogen by the reversal of reaction (3), as driven by a synthesis of ATP, is hardly feasible. This conclusion is not essentially modified by other formulations of the reactions. If the analagous calculations are made for the fermentation of glucose the following data are obtained:

(4) $$\text{glucose} + 2\ ADP^{3-} + 2\ HPO_4^{2-} \rightarrow 2\ \text{lactate}^- + 2\ ATP^{4-} + H_2O \quad (\Delta G = -27.1\ \text{Kcal})$$

Thus in the case of glucose the conditions for a synthesis by a complete reversal of the fermentation are still more unfavourable than in the case of glycogen.

Exergonic pathway from fructose-1-6-diphosphate to glycogen and glucose. Though the reversal of fermentation as a whole should thus be discounted as

* The free energy data used in this paper are taken from Burton and Krebs (31) and refer to 25°, pH 7.0; 0.2 atm. O_2; 0.05 atm. CO_2; 0.01 M concentrations of other reactants (except glycogen). The values should be taken as indicating merely the order of magnitude.

Abbreviations used in this and the following paper: ATP, ADP, AMP: adenosine triphosphate, adenosine diphosphate, adenosine monophosphate respectively. DPN^+, DPNH: oxidized and reduced forms of diphosphopyridine nucleotide (coenzyme I). TPN^+, TPNH: oxidized and reduced forms of triphosphopyridine nucleotide (coenzyme II).

a pathway of net synthesis there remains the fact that many individual steps are readily reversible, the ΔG values of these steps being low. This applies in fact to all stages except three of those in which adenosine phosphates are reactants, viz. (a) the formation of glucose-6-phosphate from glucose and ATP, (b) the formation of fructose-1-6-diphosphate from fructose-6-phosphate and ATP and (c) the transfer of phosphate from phosphopyruvate to ADP.

A pathway of carbohydrate synthesis would therefore be feasible in which some steps are those of fermentation in reverse, whilst others are special reactions, circumventing the energy barriers existing at the three stages mentioned. In cases (a) and (b) the barrier would be circumvented if in the synthetic pathway the phosphate of the hexosephosphate esters were removed by hydrolysis rather than by transfer to ATP; i.e. if the reaction

(5) fructose-1,6-diphosphate^{4-} + ADP^{3-} + H^{+} → fructose-6-P^{2-} + ATP^{4-} (ΔG = +4.3 Kcal)

were replaced by

(6) fructose-1,6-diphosphate^{4-} + H_2O → fructose-6-P^{2-} + HPO_4^{2-}

and if, furthermore, the reversal of the hexosekinase reaction

(7) glucose-6-P^{2-} + ADP^{3-} + H^{+} → glucose + ATP^{4-} (ΔG = +5.7 Kcal)

were replaced by the simple hydrolysis of glucose-6-phosphate

(8) glucose-6-P^{2-} + H_2O → glucose + HPO_4^{2-}

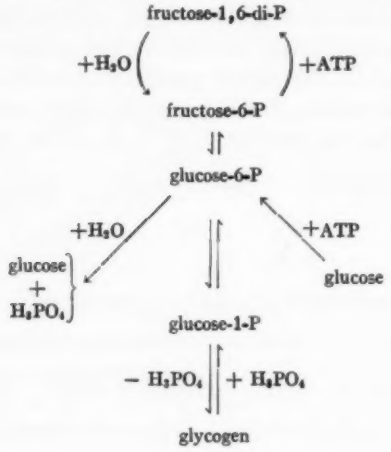
the formation of glycogen and of glucose from fructose-1,6-diphosphate then becomes exergonic. These possibilities have already been discussed by several workers, especially by Cori, Cori & Schmidt (3), Lardy (4), Swanson (5) and Cori & Cori (6). The two special enzymes postulated do in fact occur at the site of glycogen synthesis. A specific fructose-1,6-diphosphatase releasing one phosphate group of fructose-1,6-diphosphate has been discovered by Gomori (7) and studied by Roche & Bouchilloux (8) and Pogell & McGilvery (9). A specific glucose-6-phosphatase occurs in liver and has been specially studied by Fantl & Rome (10), Swanson (5) and Cori & Cori (6).

The facts are thus in agreement with the view that there is an exergonic pathway from fructose-1,6-diphosphate to carbohydrate involving the same intermediates as the reverse pathway of fermentation but employing two different reactions at the two stages where phosphorylation and dephosphorylation occur. The situation is diagrammatically illustrated by Scheme 9, where the downward arrows represent the pathway of synthesis and the upward ones the pathway of fermentation. The free-energy changes of both reactions are such as to facilitate them, ΔG of the synthesis of glycogen being -6.3 and ΔG of the fermentation being -5.0. The most powerful piece of evidence in support of this concept is the occurrence of the two specific phos-

phatases that are postulated. There are at least 9 phosphorylated intermediates, and one being a diphosphate, 10 dephosphorylating reactions are possible. Only 2 specific phosphatases have been found—the two postulated in Scheme 9.

SCHEME 9

Reactions from fructose-1,6-di-P to glycogen (downward arrows) and from glycogen to fructose-1,6-di-P (upward arrows)



Stages between lactate and fructose-1,6-diphosphate. The problem of the synthesis of carbohydrate from lactate, as far as thermodynamic feasibility is concerned, is thus reduced to that of the stages between lactate and fructose-1,6-diphosphate:

(10) $2\ \text{lactate}^- + 4\ \text{ATP}^{4-} + 2\ H_2O \rightarrow \text{fructose-1,6-diphosphate}^{4-} + 4\ \text{ADP}^{3-} + 2\ HPO_4^{2-}$ $(\Delta G = +17.8\ \text{Kcal})$

The free energy change of this overall reaction is still so unfavourable for a synthesis of carbohydrate that this reaction alone cannot cause an appreciable net synthesis. As already stated the remaining energy barrier among the individual steps is the formation of phosphopyruvate from pyruvate and ATP. Once this barrier has been overcome a synthesis of fructose-1,6-diphosphate becomes thermodynamically feasible. The relevant thermodynamic data are as follows:

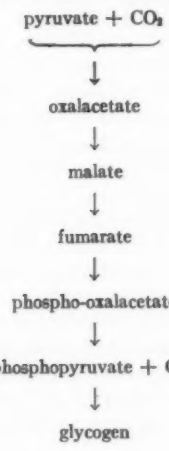
(11) $2\ \text{phosphopyruvate}^{2-} + 2\ \text{ATP}^{4-} + 2\ H_2O + 2\ \text{DPNH} + 2\ H^+ \rightarrow \text{fructose-1,6-diphosphate}^{4-} + 2\ \text{ADP}^{3-} + 2\ HPO_4^{2-} + 2\ \text{DPN}^+$ $(\Delta G = -4.4\ \text{Kcal})$

According to the ΔG values a net synthesis of carbohydrate from phosphopyruvate could occur even when the concentration of the reduced DPN is much lower than that of the oxidised form, as is usually the case.

Kalckar's experiments on phosphopyruvate formation and their interpretation. There remains thus the problem of how the energy barrier of the synthesis of phosphopyruvate from pyruvate and ATP is circumvented. Circumvention implies an alternative pathway. That such an alternative mechanism exists was first suggested by Kalckar (11) in 1939 who found a formation of phosphopyruvic acid when aqueous "extracts" (so-called) of rabbit kidney cortex were incubated with malate or fumarate, phosphate buffer and sodium fluoride. (These "extracts" were prepared by short centrifugation of 1–2 minutes of Latapie mince suspended in phosphate buffer. The supernatant was turbid and, though without cells, may well have contained mitochondria). Kalckar's suggestion of a second pathway of phosphopyruvate formation was prompted by the assumption, then generally held but now known to be incorrect, that a direct phosphorylation of pyruvate by ATP could not occur. A little later the idea of an alternative mechanism of phosphopyruvate synthesis received support when it was discovered by Hastings (12) that carbon dioxide participates in the synthesis of glycogen from lactic and pyruvic acids. Lipmann (13), and Hastings and his collaborators (14) thought that phosphopyruvate might be formed through the stages of phosphomalate or phosphofumarate, and phosphooxaloacetate. The following scheme was put forward:

SCHEME 12

Scheme of Lipmann (13) and Hastings (14) for the incorporation of CO_2 into phosphopyruvate and glycogen



The "Dicarboxylic Acid Shuttle". However this scheme was later abandoned.* In 1949 Topper and Hastings (1) stated "Following the demonstration by Lardy and Ziegler (15) that phosphorylation of pyruvate to phosphopyruvate can proceed directly in the presence of potassium ions, the necessity for postulating the formation of phosphomalate and phosphooxaloacetate as intermediates in the formation of phosphopyruvate has been removed." The fact that glycogen synthesized in the presence of lactate or pyruvate contains carbon derived from CO_2 was explained by Topper and Hastings by a reversible side reaction consisting of the carboxylation of pyruvic acid and the formation of phosphooxaloacetate. They called this series of reactions the "dicarboxylic acid shuttle" without specifying its significance, if any. Of great interest are the measurements by Topper & Hastings, with the help of C^{14}-labelled substrates, of the relative proportion of pyruvate molecules which enter the "shuttle" before phosphorylation compared with those that are phosphorylated directly. They conclude "that the dicarboxylic acid shuttle is quantitatively a very important intermediate pathway". About 4 pyruvate molecules out of 5 are not phosphorylated directly but only after "shuttling". In other words the majority of pyruvate molecules that are converted into glycogen pass through the C_4-dicarboxylic acid stage.

Lorber and his associates (16, 16a) have shown that analogous considerations also apply to the conversion of lactate and propionate into glycogen in the rat. The authors fed various isotopic forms of lactate to rats and studied the isotopic distribution in glucose prepared from glycogen. The main result that is of interest here is the conclusion that less than one-sixth of the lactate could have entered the glycogen directly by a reversal of the reactions of glycolysis. The data postulate that the bulk of the lactate passes through reactions which cause randomisation of the isotope between the α- and β-carbon

*A recent interpretation of the data is the following (Fruton and Simmonds (2) p. 457). "Lactic acid is largely removed from the blood by the liver and in this form is converted to glycogen. All the available evidence points to the initial oxidation of lactic acid to pyruvic acid followed by the reversal of the reactions of anaerobic glycolysis."

After referring to the work of Wood, and Topper and Hastings, on the distribution of C_{13} when α-labelled lactic acid is metabolised the authors state

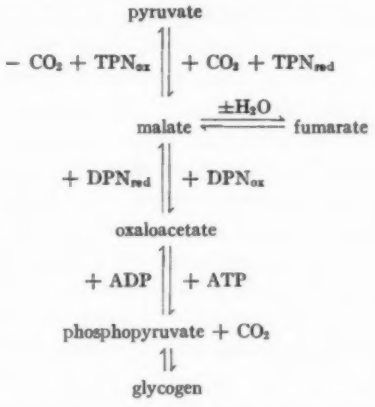
"The presence of appreciable quantities of C^{13} in the other 4 carbon atoms indicates that lactic acid or one of its metabolic products participated in other reactions which led to the observed distribution of the isotopic label. In fact, it would appear that at the most only 25 per cent of the administered lactic acid could have been converted to pyruvic acid and thus to glycogen by the direct route traced above. It must be emphasized that this conclusion does not invalidate the view that glycogen is resynthesized from pyruvic acid by a reversal of anaerobic glycolysis; it does indicate, however, that in its metabolic transformations pyruvic acid participates in other reactions which lead to the appearance of C^{13} in all of its carbon atoms. As will be seen from the discussion in the next chapter, these reactions are related to the aerobic metabolism of pyruvic acid."

atoms. A pathway visualising such randomisation would be conversion to pyruvic acid, carboxylation to oxaloacetic acid and reduction of the latter to fumaric acid. Analogous experiments with isotopic propionate likewise show that the α- and β-carbons randomise in the process of conversion into glycogen. To account for this it is again necessary to assume reactions leading to the formation of a symmetrical compound such as fumaric acid, formed either via pyruvate and oxaloacetate or via direct carboxylation of propionate to succinate.

Two pathways of phosphopyruvate formation. On general biological grounds it is most unlikely that the dicarboxylic acid shuttle is accidental and without significance. There are strong indications that the dicarboxylic acid stage is an essential step in the synthesis of glycogen, and in particular of phosphopyruvate; that the formation of the dicarboxylic acids is not the result of a "shuttle" but of a one-directional pathway involving the downward stages shown in Scheme 13. According to this scheme pyruvate and CO_2 [under the influence of the 'malic' enzyme of Ochoa (17)] forms malate, reduced TPN acting as the hydrogen donator. This is followed by the oxidation of malate to oxaloacetate, which reacts further to form phosphopyruvate, with ATP or inosine triphosphate as phosphate donators. The overall effect of these three reactions is the formation of phosphopyruvate from pyruvate.

SCHEME 13

Pathway of synthesis of phosphopyruvate



This represents an alternative pathway for phosphopyruvate formation as Kalckar had originally assumed. That Kalckar's view, contrary to what subsequent authors believed, is in fact correct can now be convincingly demonstrated. The crucial findings are as follows:

(a) Phosphopyruvate is formed in washed kidney mitochondria, i.e. in a

system which has been freed by washing from the majority of the glycolytic enzymes (18).

(b) Phosphopyruvate arises in this material only when either dicarboxylic acids or pyruvate together with bicarbonate are added. Pyruvate without bicarbonate does not react (18, 19). This is difficult to explain without the assumption that CO_2 takes part in the reaction by supplying the second carboxyl group of the dicarboxylic acid.

(c) In liver homogenates fluoride increases the yield of phosphopyruvate from dicarboxylic acids but suppresses completely its formation from phosphoglycerate, this inhibition being due to the well-established inhibition of enolase (19). This is evidence of two different mechanisms of synthesis.

(d) The synthesis of phosphopyruvate is particularly rapid in those tissues which have a low glycolytic power like liver and kidney.

The findings may be regarded as establishing the existence of a second pathway of phosphopyruvate formation, but they do not prove that the sequence of steps given in Scheme 13 involves the malic enzyme.

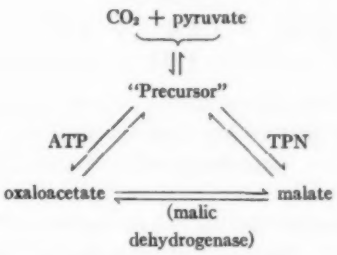
Two fixation reactions of carbon dioxide. Although the postulated reactions, on balance, proceed in one direction, each step is reversible and this implies that there are two independent reactions by which CO_2 can be incorporated into a dicarboxylic acid, the first being the reaction catalysed by the malic enzyme and leading to malate, the second the Wood-Werkman reaction leading to oxaloacetate.

Until recently it was in fact not clear whether or not two independent fixation reactions exist. That two different fixation mechanisms might exist was already suggested by the earlier work and the question whether one of the two reactions is a primary fixation reaction, or whether both occur simultaneously was first raised by Vennesland, Evans and Altman (20). They concluded that the two reactions may be independent, but that several facts point to the existence of an intimate relationship, especially the close association in the tissue of the enzymes catalysing the two reactions.

In 1950 Veiga Salles, Harary, Banfi and Ochoa (21) suggested that the reaction catalysed by the malic enzyme was the primary fixation reaction, because purified malic enzymes fixed CO_2 in oxaloacetate only when malic dehydrogenase was added. A further important contribution was made by Utter (22) who showed conclusively that pigeon liver possesses two different mechanisms for CO_2 fixation. One involves the malic enzyme and requires TPN but not ATP. The other involves oxaloacetic decarboxylase and requires ATP but not TPN. When purified extracts were incubated with pyruvate and $^{14}CO_2$ the isotope appeared in malate before it appeared in oxaloacetate provided that TPN was present. But when TPN was replaced by ATP the isotope appeared first in oxaloacetate. There remained, however, the possibility that the two mechanisms share a common initial step, a view favoured tentatively

by Utter. He suggested that the primary step in the two fixation reactions leads to a "precursor" that may either react with TPN to form malate or with ATP to form oxaloacetate. Utter's diagrammatic presentation is as follows:

SCHEME 14



The precursor in Utter's scheme may be oxaloacetate in equilibrium with pyruvate and CO_2 in the presence of oxaloacetic decarboxylase. If this assumption is correct the malic enzyme is in fact a second malic dehydrogenase, differing from the long-known DPN-specific mitochondrial malic dehydrogenase by being soluble and requiring TPN. The reduction of oxaloacetate to malate and its oxidation by the DPN specific enzyme would thus occur in different regions of the cell. A further important advance was the discovery of Utter and Kurahashi (23, 23a) that the decarboxylation of oxaloacetate by purified chicken liver extract can be completely separated from malic enzyme and requires the presence of ATP. The enzyme catalyses the reversible reaction.

$$\text{oxaloacetate} + \text{ATP} \rightleftharpoons \text{phosphopyruvate} + \text{ADP} + CO_2$$

The occurrence of the reaction may therefore be taken as an established fact. Bandurski, Greiner & Bonner (24) independently demonstrated an analogous enzyme in spinach leaves (although they refer only to the reaction as occurring from right to left). This, then, establishes the occurrence of the two reactions of Scheme 13 in which CO_2 participates. There is no need to adduce proof for the oxidative conversion of malate into oxaloacetate because the presence of malic dehydrogenase in all metabolically active tissues is a well-established fact.

Thermodynamic characteristics of phosphopyruvate formation. A point requiring further consideration concerns the thermodynamic characteristics of the phosphopyruvate formation. The standard free energy data for the individual steps are as follows:

(15) $\text{pyruvate}^- + \text{TPNH} + CO_2 \rightarrow \text{malate}^{2-} + \text{TPN}^+ \quad (\Delta G = +2.0 \text{ Kcal})$

(16) $malate^{2-} + DPN^{+} \rightarrow oxaloacetate^{2-} + DPNH + H^{+}$ ($\Delta G = +7.1$ Kcal)

(17) $oxaloacetate^{2-} + ATP^{4-} \rightarrow phosphopyruvate^{2-} + ADP^{3-} + CO_2$ ($\Delta G = -3.2$ Kcal)

The balance of these three reactions is

(18) $pyruvate^{-} + TPNH + DPN^{+} + ATP^{4-} \rightarrow phosphopyruvate^{2-} + TPN^{+} + DPNH + H^{+} + ADP^{3-}$ ($\Delta G = +5.9$ Kcal)

The equilibrium of the over-all reaction is very unfavourable for the formation of phosphopyruvate—in fact no less unfavourable than the direct reversal of the reaction catalysed by phosphopyruvate kinase. This follows from the fact that the redox potentials of the DPN and TPN systems are almost identical (25). Energy barriers, as the ΔG values of reactions 15, 16 and 17 indicate, exist at stages 15 and 16. The barrier in the case of reaction 15 can be overcome, as Ochoa, Mehler & Kornberg (17) have demonstrated by coupling with a system which decreases the concentration of TPN and increases that of TPNH. The chief TPN-linked dehydrogenase systems in liver and other tissues which could be expected to take part in a coupled reaction are the glucose-6-phosphate and phosphogluconate dehydrogenases and the isocitric dehydrogenase. I would like to defer a fuller discussion of the coupling mechanisms to the next paper as the subject is a complex one. Quantitative data on reaction rates, which I am going to mention presently, are convincing proof that some kind of coupling driving the reaction 15 effectively from left to right is available in liver tissue. There is also no difficulty in visualising a coupling of reaction 16 with an exergonic process, viz. the oxidation of reduced DPN by molecular oxygen. If the reduced DPN is continuously removed by O_2 (and oxaloacetate by reaction 17) reaction 16 proceeds rapidly from left to right.

Quantitative aspects of malate formation and removal. The evidence which I have discussed so far was concerned qualitatively with the question whether the postulated reactions can take place. It remains to be shown that the rates of the postulated component reactions are sufficiently rapid to allow the assumption that they are intermediary stages in the formation of glycogen from pyruvate.

That malate is formed in liver—pigeon liver—from pyruvate at a high rate was discovered in the Sheffield laboratory in 1939. This was part of the early work demonstrating the fixation of CO_2 in the animal. The older methods for the determination of the malate formed were intricate and only the simple method of Korkes and Ochoa (26), as elaborated by Nossal (27), has made it possible to make precise measurements of the rate of malate production in tissue preparations. This method depends on the manometric determination

TABLE I

Formation and Removal of Malate (+ Fumarate) in Pigeon Liver Homogenate
(Tissue diluted 1:30; 4 ml; 40°; 5% CO_2 in O_2; 0.005M ATP)

SUBSTRATE	0.02M PYRUVATE	0.05M FUMARATE
Change in malate + fumarate (*μmole*)		
First 10 min.	+6.82	−5.8
Second 10 min.	+4.70	−5.7
Third 10 min.	+3.34	
Malate + fumarate (first 10 min.)	+28.5	−24.2

of the CO_2 released from malate by the specific decarboxylase of Lactobacillus arabinosus. Aerobically the rate of malate formation from pyruvate in homogenised pigeon liver is very high at the initial stages of incubation. The increase in the malate concentration is not proportional to time and in fact in the later stages, which according to the conditions, may start as early as 10 minutes, the malate concentrations becomes stationary or decreases. This is due to the fact that malate is rapidly oxidised in these homogenates, the rates of oxidation being of the same order as the rates of formation. In terms of the Q-nomenclature values between 20 and 30 are obtained for both rates. Sample experimental data are given in Table I. Owing to the high activity of fumarase, any malate formed is partly converted into fumarate. The sum of malate + fumarate is therefore given. The data show, to sum up, that the rates of formation and of removal of malate are very high.

Fate of malate. The question arises of what happens to the malate that is oxidised. As the rates of malate removal are of the same order as (generally a little higher than) the rate of oxygen uptake, the malate removed cannot be completely oxidised. Bartley and Avi-Dor (28) have identified phosphorylated 3-carbon compounds as products of the incomplete oxidation. Phosphopyruvic and 3-phosphoglyceric acids appear in relatively large quantities, under some conditions even quantitatively. The yields are large, up to 60 per cent, when washed mitochondrial preparations are used instead of cell suspensions containing all tissue components. The reason is that phosphopyruvate is formed by the enzyme systems of the insoluble fractions but removed by those of the soluble fractions.

Phosphopyruvate and phosphoglycerate are also formed anaerobically when oxaloacetate is added (together with ATP and fluoride) but relative high concentrations of the substrate are required (28).

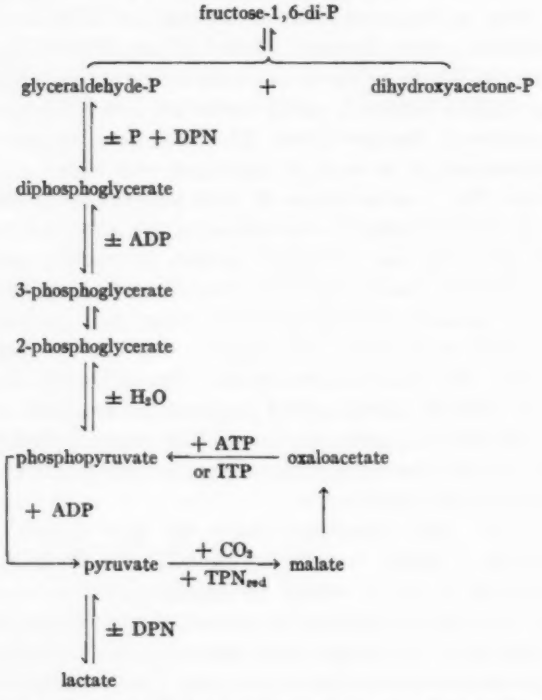
Pathway of carbohydrate synthesis. To sum up, the postulated reactions leading to the formation of phosphopyruvate have all been demonstrated to occur. This establishes the possibility of the mechanism. That carbohydrate is actually formed through the postulated sequence of reactions is clinched by two facts already quoted, namely the participation of CO_2 in the synthesis of carbo-

hydrate from lactate and pyruvate and the randomisation of the α- and β-carbon atoms of lactic acid. The latter fact implies that these two carbon atoms must at one stage be part of a symmetrical molecule. The scheme advanced makes provision for this, the symmetrical molecule being fumaric acid formed by a very rapid side reaction from malate.

The picture of the pathway of carbohydrate synthesis from noncarbohydrate sources which emerges exhibits a remarkable feature. Every intermediate carbon compound of glycolysis also occurs in the synthesis, but the reactions by which the intermediates are formed differ at three points. This is illustrated by Schemes 9 and 19. In two cases phosphate is removed by hydrolysis in place of transfer to ADP (Scheme 9). In the third a pathway employing two new intermediates—malate and oxaloacetate—is employed (Scheme 19).

SCHEME 19

Pathways of interconversion of fructose-1,6-diphosphate and lactate. The pathways of fermentation and resynthesis differ only in the stages between pyruvate and phosphopyruvate



Another way of describing the situation is to say that cyclic systems, (i.e. systems which are not thermodynamically reversible but where the inter-

conversion of the products involves different mechanisms for the backward and forward reactions) are inserted in the glycolytic chain of reactions.

The significance of the special steps in the pathway of synthesis lies in the fact that they provide facilities for coupling with exergonic reactions, e.g. the coupling of the malic enzyme through TPN with the dehydrogenation of glucose-6-phosphate or isocitrate. The overall chemical changes associated with the synthesis of carbohydrate (which should include the coupled reactions) cannot yet be precisely stated, but the principle is clear. The endergonic conversion of lactate into glucose is associated with, say, the oxidation of isocitrate to α-ketoglutarate; the oxidation of malate by molecular oxygen and the hydrolysis of additional ATP (forming ADP in the Utter-Kurahashi reaction and releasing the phosphate later when fructose-1,6-diphosphate is partially hydrolysed).

That the synthesis of glycogen is not a complete reversal of glycolysis has clearly been stated before. Cori, Cori & Schmidt (3), Lardy (4), Swanson (5) discussed the importance of dephosphorylation in place of phosphate transfer to ADP in the synthetic pathway. The assumption that the malic enzyme cycle, involving the formation of malate and oxaloacetate as intermediates, is part of the mechanism of carbohydrate synthesis has recently been ventilated by Shreeve (19). There has been no clear suggestion previously about the physiological significance of the malic enzyme. The wide-spread occurrence of this enzyme in plant material, especially at the site of carbohydrate synthesis, suggests that the role of this enzyme system in plants is the same as in the animal. In fact Vickery and his associates (30) have observed that carbohydrate synthesis in the dark in excised leaves of Bryophyllum was paralleled by the disappearance of malic acid; this agrees with the assumption that malate is a precursor of the carbohydrate, but of course does not prove it.

The concept also throws fresh light on the role of the Wood-Werkman (or Utter-Kurahashi) reaction, on the "shuttle" of Topper and Hastings (which no longer appears as a to-and-fro pendulum leading nowhere but as a one-directional pathway in the synthesis of carbohydrate), on some aspects of the Pasteur effect and the value of the Meyerhof quotient. A number of experiments suggest themselves as further tests of the validity of the hypothesis. Can malate be converted into carbohydrate at least as rapidly as lactate or pyruvate? Does the location of the malic enzyme coincide with the site of carbohydrate synthesis?

I started this paper with some general remarks on biological syntheses and on the question whether or not they are the reversal of degradation. It is probable that the pattern of carbohxdrate synthesis from non-carbohydrate precursors reflects a general principle, namely the inclusion in the pathway of synthesis of special steps which do not occur in the degradation—steps where coupling with exergonic reactions takes place.

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SOME ASPECTS OF THE METABOLISM OF ADENOSINE PHOSPHATES*

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Received for publication May 3, 1954

I. COUPLING OF MALATE SYNTHESIS WITH TPN-SPECIFIC DEHYDROGENASE SYSTEMS

In the course of my first paper I mentioned the rapid conversion of pyruvate and CO_2 into malate in avian or mammalian liver, a reaction which is known to be dependent on the presence of TPN and which has been formulated as follows:

$$(1) \qquad \text{Pyruvate}^- + \text{TPNH} + CO_2 + H^+ \rightarrow \text{malate}^{2-} + \text{TPN}^+ \qquad (\Delta G = +2.0 \text{ Kcal})$$

In view of the endergonic nature of this reaction the question arises of the coupling with exergonic reactions which enables the reaction to give large net yields of malate. As I have already stated, several TPN-linked dehydrogenase systems are present in liver and might be expected to take part in a coupled reaction, such as glucose-6-phosphate and 6-phosphogluconic dehydrogenases, or isocitric dehydrogenase.

As the coupling of dehydrogenase systems through TPN (or DPN) does not require molecular oxygen the coupling is best tested experimentally under anaerobic conditions. The malate and fumarate formed do not disappear in liver homogenates under these conditions and the increase in the amounts of malate formed from pyruvate on addition of substrates that can donate hydrogen to TPN may be regarded as a direct indication of a coupled oxido-reduction.

As shown in Table I citrate and glucose-6-phosphate cause substantial increases in the yield of malate and fumarate whilst glutamate and lactate have no effect. However the rate of malate formation after addition of citrate and glucose-6-phosphate is still very low, no more than 15 per cent of the rate of aerobic formation of malate. This suggests that the anaerobic linking of two dehydrogenase systems through TPN is not the only mechanism which provides the energy for the reductive synthesis of the malate from pyruvate and CO_2. There are other observations—experiments with dinitrophenol (DNP)—which suggest that another coupling mechanism is responsible for the high aerobic rate of malate formation.

* Lecture II of The Herter Lectures delivered at The Johns Hopkins Hospital February 25, 1954.

TABLE I

Effects of Various Substrates on the Anaerobic Formation of Malate from Pyruvate

(4 ml. pigeon liver homogenate (1:20); 0.02M pyruvate; 5% CO_2 in N_2. 40°. 40 min.)

ADDITIONAL SUBSTRATE	MALATE FORMED	Q_{Malate}
	(*μmole*)	
—	1.3	+0.9
citrate	5.1	+3.4
glucose-6-P	5.0	+3.3
glutamate	1.1	+0.7
DL-lactate	1.2	+0.7

TABLE II

Effect of Dinitrophenol on the Anaerobic Formation of Malate from Pyruvate

(4 ml. pigeon liver homogenate (1:20); 40°; 40 min.; 5% CO_2 in N_2)

SUBSTRATES	PYRUVATE + CITRATE		PYRUVATE + GLUCOSE-6-P	
Dinitrophenol	0	10^{-4}M	0	10^{-4}M
Malate formed (μmole)	8.27	7.45	3.53	2.99

TABLE III

Aerobic Formation of Malate from Pyruvate in Pigeon Liver. Effect of Dinitrophenol

(0.02M pyruvate; 4 ml. homogenate 1:20; 40°)

	MALATE FORMED (*μmole*)	
	No DNP	10^{-4}M DNP
min.		
5	5.3	2.5
10	8.1	0.4
20	10.3	1.0

Effects of dinitrophenol. At low concentrations this agent does not interfere with coupled oxido-reductions and it is therefore no surprise to find that the *anaerobic* formation of malate from pyruvate is not affected by DNP (see Table II). In contrast DNP is a powerful inhibitor under aerobic conditions. As Table III shows there is a 90 per cent inhibition after 20 min. (the lower inhibition at the earlier stages is probably due to the anaerobic component operating at the early times of incubation whilst endogenous hydrogen donators are available). The degree of inhibition depends on the presence of additional substrates (Table IV), again because some substrates react anaerobically through a coupled oxido-reduction which is DNP-insensitive.

DNP-sensitivity indicates involvement of adenosine triphosphate in the formation of malate and this conclusion raises the question of how participation of ATP can be visualised. The synthesis of malate from pyruvate requires reduced TPN, and it has been pointed out by Davies and Ogston (1) and by Davies and Krebs (2) that it is possible to visualize a coupling between the

TABLE IV

Effect of Dinitrophenol on the Aerobic Formation of Malate in the Presence of Various Substrates

(4 ml. pigeon liver homogenate (1:20); 0.02M pyruvate; 5% CO_2 in O_2)

ADDITIONAL SUBSTANCES	MALATE FORMED (*μmoles*)	
	5 min.	10 min.
—	4.6	6.3
citrate	6.1	8.1
citrate, DNP(10^{-4}M)	2.6	2.7
glutamate	5.6	6.9
glutamate, DNP(10^{-4}M)	0.6	0.2

hydrolysis of ATP and the reduction of pyridine nucleotides; in other words ATP—paradoxically though it may appear at first—can bring about a reduction.

ATP as a reducing and oxidising agent. The oxidation of reduced TPN may be formulated as follows:

$$TPNH + H^+ + O \rightarrow TPN^+ + H_2O$$

If this reaction is analogous to the oxidation of DPN (which remains to be proved) it may be coupled with the synthesis of ATP from ADP in the following manner

$$(1) \quad TPNH + H^+ + \text{flavoprotein} + ADP + P \rightarrow TPN^+ + H_2O + \text{dihydroflavoprotein} + ATP$$

If, furthermore, the reaction were reversible (which has not yet been demonstrated in the reference case of DPN) then ATP (through the intermediation of reduced flavoprotein) could reduce TPN, because in a reversible system addition of ATP shifts the interconversion of the two forms of TPN in favour of the reduced form. As ATP can be generated by a variety of biological oxidations, it may represent a general link between any energy-giving, i.e. ATP synthesizing reaction, and the reductive formation of malate from pyruvate and CO_2.

That ATP can in fact function as a reducing agent is already well established for another system. This is the glyceraldehyde phosphate dehydrogenase system in which the oxidation of the glyceraldehyde phosphate is coupled with the synthesis of ATP:

$$\begin{array}{l} CH_2 \cdot O \cdot PO_3H_2 \\ | \\ CHOH \\ | \\ HCO \end{array} + H_3PO_4 + DPN^+ + ADP \rightleftharpoons \begin{array}{l} CH_2O \cdot PO_3H_2 \\ | \\ CHOH \\ | \\ COOH \end{array} + DPNH + ATP + H^+$$

The coupling mechanism in this reaction—a key stage of anaerobic glycolysis—is well understood. The equilibrium of the system is known from measurements of Bücher (3). It is in favour of the right but owing to the reversibility of the reaction the addition of ATP to the equilibrium mixture results in a reduction of a carboxylic acid to the aldehyde. It should be noted that in this case the hydrogen required for the reduction is supplied by reduced DPN which in turn undergoes oxidation. Thus ATP can cause reduction as well as oxidation, and in fact these are bound to be always coupled. It would therefore be more correct to say that ATP can shift equilibria in oxidation-reduction systems; in the glyceraldehyde phosphate dehydrogenase system its addition causes oxidation of DPN and reduction of phosphoglyceric acid. In the hypothetical TPN system considered previously it is dihydroflavoprotein which undergoes oxidation and TPN which undergoes reduction.

To sum up the gist of these considerations:

(a) the increased formation of malate from pyruvate in the presence of oxygen indicates that TPN can be reduced by a mechanism that is different from the action of a simple dehydrogenase system.

(b) the inhibition of the effect of oxygen on the reductive formation of malate indicates the participation of ATP.

(c) ATP is known to be capable of shifting equilibria in oxido-reduction systems and a reduction of pyridine nucleotide under the influence of ATP, though not proven, is feasible.

II. A SPECIAL FEATURE OF ATP AS AN ENERGY STORE

In what I have already said there is implied a property of ATP which deserves elaboration: energy-rich phosphate bonds are synthesized at the expense of the free energy of oxido-reductions irrespective of the level of the redox potential of the systems concerned. As long as the two interacting systems have suitable concentrations and redox potentials to yield sufficient free energy on interacting they can supply energy for phosphate-bond synthesis. The fact that the release of energy from pyrophosphate bonds is independent of the redox scale allows phosphate-bond energy to be used as an "energy currency" in any situation. For example, ATP may be formed either by a reaction of the type formulated in (1), or by the reaction

(2) dihydroflavoprotein + 2 ferricytochrome c + ADP + P →
flavoprotein + 2 ferrocytochrome c + H_2O + ATP

The pyrophosphate bond of ATP in living systems can thus act as an energy link between different oxido-reduction systems. This is analogous to the action of electrons in inorganic systems which allow one oxido-reduction cell to drive another cell backwards, even though the components of this cell may have lower oxido-reduction potentials and would be oxidised by the components

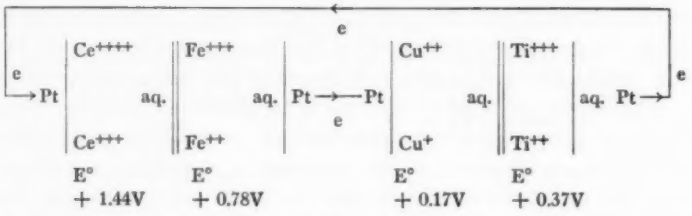

FIG. 1. Oxidation-reduction cells coupled by wire. Reduction (of Cu^{++}) is caused by a powerful oxidizer (Ce^{++++}).

of the first cell if they were mixed. In the electric cells shown in Fig. 1 the reduction of Ce^{++++} leads to the *reduction* of Cu^{++}, although Ce^{++++} is a much stronger oxidizing agent.

It is no doubt of great significance that in a system which derives its energy in the last resort from oxido-reductions the free energy of the hydrolysis of ATP is independent of redox potentials. Like the electrons in the platinum wire ATP can act as energy currency and link energetically systems of widely different redox potentials.

III. GENERATION OF PYROPHOSPHATE BONDS OF ATP

I now propose to discuss the arrangement of metabolic processes which play a part in the formation of ATP in living matter. In the last resort the pyrophosphate bonds are synthesized at the expense of the free energy of the degradation of foodstuffs. Early workers who studied the fate of foodstuffs were often struck by the complexity of these reactions, especially of the oxidative mechanisms, where a sequence of three types of catalysts—the pyridine nucleotides, flavoproteins and iron-porphyrins—participate. The appreciation of the role of ATP has contributed a great deal in simplifying the overall picture of the energy-producing mechanisms (4).

The three phases of foodstuff degradation. The production of energy by the combustion of foodstuffs in living matter may be said to proceed in three major phases (see Table V). In Phase I the large molecules of the food are broken down to the small constituent units. Proteins are converted to amino acids, carbohydrates to hexoses and fats to glycerol and fatty acids. The amounts of energy liberated in these reactions are relatively small, of the order of 0.5 per cent of the total free energy that can be released in the breakdown of polysaccharides and proteins and no more than 0.1 per cent in the case of polysaccharides and triglyceride fats. These quantities are not utilised, except for the generation of heat. The reactions of this first phase thus do not yield utilisable energy: they merely prepare foodstuffs for the energy-giving processes proper. They take place in the intestinal tract and also in tissues when reserve material is mobilised for energy production.

TABLE V

The Three Main Phases of Energy Production from Foodstuffs

PHASE	OUTLINE OF CHEMICAL CHANGE
I	carbohydrates → hexoses proteins → about 20 amino acids fats → glycerol; fatty acids
II	hexoses → lactic acid → pyruvic acid glycerol → pyruvic acid several amino acids (alanine, serine, cysteine) → pyruvic acid fatty acids → acetyl-coenzyme A several amino acids (3 leucines, tyrosine, phenylalanine) → acetyl-coenzyme A several amino acids (glutamic acid histidine, prolines, arginine) → α-ketoglutaric acid several amino acids → oxaloacetic acid
III	acetyl-coenzyme A + α-ketoglutaric acid → tricarboxylic acid cycle oxaloacetic acid → tricarboxylic acid cycle

In Phase II the diversity of small molecules produced in the first phase—three or more different hexoses, glycerol, about twenty amino acids and a number of fatty acids—are incompletely burned, the end-product being, apart from carbon dioxide, one of three substances: acetic acid in the form of acetyl coenzyme A, α-ketoglutarate, or oxaloacetate. The first of these three constitutes the greater amount: two-thirds of the carbon of carbohydrate and of glycerol, all carbon atoms of the common fatty acids and approximately half the carbon skeleton of amino acids yield acetyl coenzyme A. α-Ketoglutarate acid arises from glutamic acid, histidine, arginine, citrulline, ornithine and proline; oxaloacetic acid from aspartic acid and, through malic acid, from part of the benzene ring of tyrosine and phenylalanine. The details of the pathways cannot be discussed here in full, but one matter of principle should be emphasised: the number of steps which living matter employs in order to reduce a great variety of different substances to three basic units is astonishingly small and could certainly not be equalled with the tools at present available to the organic chemist.

The three end-products of the second phase are metabolically closely interrelated. They take part in the Phase III of energy production: the tricarboxylic acid cycle, the common "terminal" pathway of oxidation of all foodstuffs.

As each step of the intermediary metabolism requires a specific enzyme it is evident that a common pathway of oxidation results in an economy of chemical tools. Surveying the pathway of the degradation of foodstuffs as a whole one cannot but be impressed by the relative simplicity of the arrangement. The total number of steps required to release the available energy from a multitude of different substrates is unexpectedly small.

The energy yielding steps of intermediary metabolism. But the economy of stages does not end here. Energy is not released at every intermediary step of metabolism. Most though not all of the available energy contained in the foodstuffs is liberated when the hydrogen atoms removed by dehydrogenations react with molecular oxygen to form water. Such reactions in which 40 to 60 kcal. are liberated per mole occur at Phase II and at Phase III. Roughly one-third of the total energy of combustion is set free at Stage II and two-thirds at Phase III.

Even with a common terminal pathway of oxidation and the shortness of the routes at Phase II, the number of stages where utilisable energy is set free is still considerable. There are four such stages in the tricarboxylic acid cycle. At Phase II there are a large number, over twenty, different oxidative reactions (Table VI). Their number cannot be precisely stated as the pathway of oxidation of some amino acids (e.g. tryptophane, methionine, lysine) is not yet known in all detail. There are two oxidative stages each when carbohydrates or when fatty acids are prepared for entry into the tricarboxylic acid cycle. One special stage is required for glycerol, two others being identical

TABLE VI

Stages of Intermediary Oxidations Where Major Quantities of Energy (40–60 Kg. cal./mole) Are Liberated

1. *Tricarboxylic acid cycle*
   1. isocitrate → oxalosuccinate
   2. α-ketoglutarate → succinate
   3. succinate → fumarate
   4. L-malate → oxaloacetate
2. *Carbohydrate breakdown*
   5. lactate → pyruvate
   6. pyruvate → acetyl coenzyme A
3. *Fatty acid breakdown*
   7. fatty acid → β-hydroxy acid
   8. β-hydroxy acid → β-keto acid
   9. glycerophosphate → dihydroxyacetone phosphate
4. *Amino acid breakdown*
   10. α-amino acid → α-keto acid (16 different amino acids)
   11. proline, arginine → glutamic acid (2 stages each)
   12. tyrosine, phenylalanine → malic and aceto acetic acids (4 stages)

with those of carbohydrate. Amino acids, with a few exceptions, require at least one oxidative step each—the oxidative deamination. In the cases of glutamic acid, aspartic acid, and alanine this one step is sufficient to produce an intermediate also encountered in either carbohydrate breakdown or the tricarboxylic acid cycle. The exceptional amino acids referred to above, which do not require special oxidative steps, include histidine which yields glutamic acid anaerobically, and cysteine and glycine, which are both convertible into serine. In many other cases additional steps are required. Some data about the number of energy releasing steps in Phase II of amino acid metabolism are given in Table VI. There are altogether between 20 and 30 stages at which major parcels of energy are liberated. This is already a relatively small number considering the variety of starting materials but by some simple devices the number receives a further drastic reduction.

The oxidative stages in which molecular oxygen is the final oxidising agent are all complex reactions which consist of at least four different stages involving the pyridine nucleotide and the flavoproteins. This is illustrated by Table VII for the case of the oxidation of lactate to pyruvate. The quantitative aspects of the free energy changes associated with the different stages are shown in Table VIII. The main feature is that no major quantities of energy are set free at the first step when the pyridine nucleotide reacts with the substrate, except when α-ketonic acids are oxidised, a case to which I shall refer again later. The free energy of the oxidation of the substrate is liberated, as Ball (5) has first clearly realised, when the reduced forms of pyridine nucleotide are reoxidised by molecular oxygen through the stages shown in Table VII.

These stages are common to the great majority of the oxidative steps. An

TABLE VII

Stages in the Biological Oxidation of Lactate

Lactate + pyridine nucleotide → pyruvate + dihydropyridine nucleotide
dihydropyridine nucleotide + flavin nucleotide
→ pyridine nucleotide + dihydroflavin nucleotide
dihydroflavin nucleotide + 2 ferricytochrome
→ flavin nucleotide + 2 ferrocytochrome + $2H^+$
2 ferrocytochrome + $\frac{1}{2}O_2$ + H_2O → 2 ferricytochrome + $2OH^-$

Lactate + $\frac{1}{2}O_2$ → pyruvate + H_2O

TABLE VIII

Standard Free Energy Changes in the Transport of Electrons or Hydrogen Atoms from Substrate to O_2

Substrate	
↓	ΔG small, variable
pyridine nucleotide	
↓	ΔG −11 Kg.cal.
flavin nucleotide	
↓	ΔG −16 Kg.cal.
iron porphyrin	
↓	ΔG −25 Kg.cal.
O_2	

exception is the oxidation of succinic acid which does not require a pyridine nucleotide or flavoprotein but is catalysed by iron porphyrins only. The exceptional position of the succinic dehydrogenase system is conditioned by thermodynamic characteristics of this system whose oxidation potential system is less negative than that of the other substrate dehydrogenases.

Thus the energy release in the oxidation of different substrates by molecular oxygen generally occurs essentially through the same series of reactions, independent of the nature of the substrate. A major exception appears to be the deamination of amino acids but this exception is probably an apparent one. One amino acid—glutamic acid—can actually interact with pyridine nucleotide and virtually all amino acids can transfer their amino group to α-ketoglutarate by transamination. Transamination links the deamination of all amino acids with the dehydrogenation of glutamic acid by pyridine nucleotide (Table IX). Whilst this is not the only mechanism of deamination it probably represents a major one. If this is true the free energy of the oxidation of amino acids, like that of other substrate oxidations, would be released along the standard electron-carrier chain. It is an attractive aspect of this concept that it ascribes biological significance to transamination enzymes, the metabolic role of which is otherwise not understood.

To sum up, the energy-yielding oxidative stages are all complex reactions which involve (a) a specific primary step, or series of steps, namely the

TABLE IX

Deamination of Amino Acids

amino acid + α-ketoglutarate $\rightleftharpoons$ keto acid + glutamate (transamination)
glutamate + pyridine nucleotide $\rightleftharpoons$ α-ketoglutarate + NH_3 + reduced pyridine nucleotide

TABLE X

Energy Giving Reactions Proper

2 stages of anaerobic glycolysis
3 stages of oxidation of reduced pyridine nucleotide
α-keto acid + pyridine nucleotide $\rightarrow$ carboxylic acid + CO_2 + reduced pyridine nucleotide
succinate + 2 ferricytochrome C $\rightarrow$ fumarate + 2 ferrocytochrome C

reaction with pyridine nucleotide and, in the case of amino acids, transamination (b) a set of intermediary hydrogen carriers, reacting as indicated in Table VII, and common to all major stages. The release of energy occurs along this common pathway and not when the substrate reacts with pyridine nucleotide. This arrangement reduces the total number of energy-yielding steps to seven, or more correctly, to seven types. These are listed in Table X. Two occur in the anaerobic glycolysis. Three more occur, as already mentioned, when the reduced pyridine nucleotide is oxidised by molecular oxygen, through the intermediation of flavoproteins and iron-porphyrins. The sixth energy-giving reaction proper is the dehydrogenation of α-ketonic acids by DPN. This is the only known type of reaction between a substrate and DPN where sufficient quantities of free energy arise to permit the synthesis of a pyrophosphate bond. The seventh reaction is the oxidation of succinate by cytochrome c. As already stated succinate, owing to its unusual thermodynamic characteristics, has a special place among the common substrates and intermediates of oxidation.

The crucial feature of these seven reactions, from the biological point of view, is not the liberation of energy, but the coupling of energy production with energy utilization, i.e., with the synthesis of pyrophosphate bonds of adenosinetriphosphate. The utilization of the free energy of a chemical process requires a coupling mechanism specifically adjusted to the circumstances of the energy-yielding steps. By the reduction of the number of such steps to a minimum, living matter reduces the requirement for specific mechanisms and thereby effects a striking economy.

The mechanism of energy transfer is known for two of the seven energy-giving reactions—those of anaerobic glycolysis. For another, the oxidative decarboxylation of α-ketonic acids, it is known in principle but some uncertainty of detail remains. It is completely unknown for the remaining four reactions.

Before leaving the subject I would like to emphasize that many phenomena in the chemical make-up of living matter, the significance of which has baffled earlier investigators, appear in a new light when they are considered as parts of one organised energy-producing mechanism. They facilitate in an economical way the utilisation of the free energy of foodstuff degradation for the manufacture of the one fuel on which the working of energy-requiring mechanisms of living matter rests, the pyrophosphate bonds of ATP. The participation of three types of oxido-reduction catalysts in the interaction between molecular oxygen and substrate, at first perhaps appearing as an unduly complicated arrangement, is essential in that it breaks up a large energy parcel of 50 kcal. into three smaller ones, each of a size suitable for an effective generation of one pyrophosphate bond.

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EXCURSION INTO THE BORDERLAND OF BIOCHEMISTRY AND PHILOSOPHY*

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Received for publication May 3, 1954

I. TELEOLOGICAL CONSIDERATIONS IN BIOLOGY

My starting point for this excursion into the borderland of biochemistry and philosophy is the factual picture of energy production in living matter, i.e. of ATP synthesis, that I discussed in my second paper.

A striking feature of this picture is the relative simplicity of the basic principles. In spite of the great variety of ultimate sources of energy—several hexoses, some twenty amino acids, several fatty acids and glycerol—and in spite of the many stages through which each of these 30 or so substances must pass in the process of degradation, there are in higher animals only seven types of energy-giving reactions proper where major energy parcels are set free and where ATP is synthesized from ADP and inorganic phosphate. Moreover the stages of degradation are unexpectedly small in number, owing to a common terminal pathway for all three major foodstuffs and owing to the shortness of the route that leads to this common pathway.

In discussing energy production I have tacitly and sometimes openly (but without emphasizing it) made the assumption that the arrangement of the energy producing mechanism is purposeful: for example, that it is the primary purpose of the oxidative degradation of foodstuffs to generate phosphate bond energy; that the small number of energy giving reactions proper presents an economy of chemical tools (the term 'economy' implying the purpose of saving); that transaminases have the significance or purpose of canalising the free energy changes of the deamination of amino acids towards the electron carrier chain. I now propose to examine the epistemological justification of "teleological" considerations in this context.

Some writers reject teleology wholesale as "unscientific". To E. W. von Brücke is attributed the remark "Teleology is a lady without whom no biologist can live. Yet he is ashamed to show himself with her in public". This phrase describes picturesquely the frame of mind of some scientists from Brücke's days (1819 to 1892) to the present time (1, 2, 3).

It is my view that the wholesale dismissal of teleological consideration in biology is unjustified and rests on misunderstandings. It is not sufficiently

*Lecture III of The Herter Lectures delivered at The Johns Hopkins Hospital February 26, 1954.

realised that there are several forms or shades of teleological reasoning. Whilst several of these are certainly unscientific others are not only admissible but necessary in biological thought. All kinds of teleology are out of place in physics, astronomy, chemistry and all other physical sciences where nothing is gained by asking the question, as did Aristotle and his followers up to the times of Descartes and Bacon, "What purpose does this event serve?". In the physical sciences only the question, "What circumstances caused this event?", leads to scientific knowledge. Nor is there any justification, as Kant made clear in his Critique of Judgement, for that naive form of teleology contained in the "physiotheological proof" of the existence of God.

A very different matter are teleological considerations in biology. The properties of living matter can never be fully explored and truly understood without making reference to some ends which they serve. The special position of biology has been stated by many writers such as J. S. Haldane (4).

> "The very nomenclature of biology embodies the conception that life, in whatever form it may occur, occurs as a specific whole, in which the parts and actions are essentially related to one another, and cannot be isolated without destroying their nature. The working hypothesis of biology is that this wholeness exists, and this working hypothesis has carried biology forward just as successfully as the Newtonian conception has carried the physical sciences forward. Biologists are, and always have been, progressively tracing the specific co-ordination which shows itself in the structure, activities, and environment of living organisms. This co-ordination cannot be expressed in terms of ordinary physical and chemical conceptions. For this reason biology must be regarded as a distinct science or group of sciences. A biologist interprets his observations in a different manner from that of a physicist."

The idea put forward in this quotation has been, at least on principle, accepted by all modern writers who have searched into the epistemological basis of biological science (5, 6, 7, 8). To illustrate this, two passages from Cassirer's book (6) may be given. The first refers to Ungerer's views, the second to Kant's.

> "To employ a teleological method in the study of living organisms means only that we examine the processes of life so as to discover to what extent the character of preserving wholeness manifests itself. This is essential for scientific knowledge and is fully adequate to it." (p. 213).
>
> "The concept of purpose can never be struck out of the whole of natural knowledge and absorbed into the idea of cause. For even if it is not an independent principle for the explanation of nature, still the approach to one of her most important domains would be barred without it, and the knowledge of phenomena would therefore be incomplete and defective. Kant limited the principle of purpose to this role of taking cognizance of nature." (p. 122).

The last quotation states an important limitation in the scientific use of teleology. It cannot be adduced to explain matters but may be used to formulate questions, which guide the research worker as a working hypothesis. When the biologist asks "What purpose does this event serve?", he does not argue that the event is caused by the purpose it serves.

It is evident that anyone who is faced with a man-designed machine which is not familiar to him will be greatly helped in the examination of the machine if at each stage he raises the question of the purpose of the component parts. This will assist him in understanding the working of the machine; and just as the teleological viewpoint in biology does not "explain" how a purposeful phenomenon has arisen, so the understanding of the purpose of the man-made machine will not necessarily reveal how it was built.

Much of the confusion about teleology, as Cassirer points out, has arisen from misunderstandings of the role of teleology in evolutionary theory. Some followers of Darwin, in particular Haeckel, thought that Darwinism had disposed of all teleological considerations in biology. Cassirer (6) summarizes Haeckel's views as follows:

"The doctrine of descent gives us not only a detailed acquaintance with all the phenomena of life; but in addition a satisfactory answer to every question regarding the 'why' of these phenomena. And these answers are of a purely mechanical and causal nature. They show that natural, physico-chemical forces alone are the causes of phenomena which had been customarily ascribed to the immediate influence of supernatural creative powers. It was this elimination and overcoming of all teleological considerations that Haeckel believed to be Darwin's great act of emancipation, one comparable with the achievement of Copernicus. Now for the first time there was feasible a treatment of nature from which all anthropomorphic features had been stripped away. The idea of purpose had been driven out of that last refuge from which even Kant's critique had been unable to dislodge it. The 'Newton of the grass blades' whose possibility Kant had doubted, had appeared at last in Darwin, and his theory of selection had solved the riddle that Kant had believed insoluble." (p. 162).

Cassirer proceeds to expose the fallacy of these views:

"That Darwinism has finally overcome the purposive way of looking at things, that it has succeeded in dispensing with all concepts of purpose in scientific research, is something which cannot be asserted if one even attends only to its most general logical structure. Here the concepts of purpose have their secure place; they prove themselves to be not only admissible but absolutely indispensable. Not only the answer that Darwinism yields but even its formulation of the problem is indissolubly bound up with the purposive idea. The concepts of 'fitness', 'selection', 'struggle for existence', 'survival of the fittest'—these all have plainly a purposive character and they exhibit a theoretical organization quite different from that of the concepts of the mathematical sciences. The constant use that Darwinism makes, and must make, of these concepts would alone suffice to show that in opposing a definite form of metaphysical teleology it in no way renounces 'critical teleology'.

"Indeed, one can go even further and assert that no earlier biological theory ascribed quite so much significance to the idea of purpose, or advocated it so emphatically, since not only individual but absolutely all the phenomena of life are regarded from the standpoint of their survival value. All other questions retreat into the background before this one." (p. 166).

Haldane (4) and von Bertalanffy (7) prefer to speak of "wholeness" rather than of "purposiveness" of living organisms, when referring to the phenomenon that the great majority of, if not all, biological processes are directed towards the preservation, repair or restitution of the whole organism. This distinction, however, is hardly a major one.

In order to avoid a teleological terminology Huxley (9) uses the expression "biological improvement" when dealing with progress in evolution. Improvement is defined as an increase in efficiency, i.e. "the business of existence and survival," in other words the standard by which improvements are judged is their usefulness to the organism as a whole.

Others consider the term "function" more respectable than the term "purpose". I do not think that anything is gained by avoiding straight teleological terms. What is essential is that it is appreciated that we expect the components of living matter to have a function that is useful towards the maintenance of life. Respectability is not restored by adopting another name for the same thing but by exonerating the principle in question.

It should perhaps not be claimed that the "proof" of the "wholeness" and "purposiveness" of living matter can be generally established in the same way as experimental findings. The place of these concepts in the scientist's world is at a different level. At the foundation of all sciences, as Born (10) has put it, there are "fundamental assumptions which cannot be further reduced, but have to be accepted by an act of faith". Born's remark, "Even an exact science like physics is based on fundamental beliefs", echoes an earlier sentence of Planck (11). "Auch in der Physik gilt der Satz, dass man nicht selig werden kann ohne den Glauben". The wholeness and purposiveness of living matter is one of the fundamental principles which biologists must accept, or reject, as an act of faith, though of faith, as Haldane (4) remarks, firmly based on experience. If they choose to reject it they deprive themselves not of a somewhat disreputable entertainment, as von Brücke's phrase implies, but of a legitimate and powerful tool.

In support of the view I can also quote Niels Bohr (12). "The existence of life must be taken as a basic fact for which no specific reason can be given and which must be accepted as the starting point of biology, in the same way in which the quantum, together with the existence of the elementary particles form the basis of physics".

Bohr specifically states that teleological considerations, though they have no place in physics, may be applied to certain problems presented by living matter. What are these "certain problems"? The concept of wholeness is required because the properties of living matter can never be fully explored and truly understood without making reference to some ends which they serve.

It does not detract from the value of the teleological viewpoint in biology that it does not prove useful in every case. On the basis of current evolutionary theory, it is not to be expected that every property of living matter is necessarily purposeful. Fitness required for survival is not necessarily identical with perfection. In the course of evolution mutations are bound to arise which are neither harmful nor beneficial and these may persist for many generations.

Thus, any species is liable to carry some chance characteristics which do not contribute to the "wholeness" of the organisms. They are "ballast", marring the perfection of the organisation of living matter. As examples of properties which baffle the attempts to account for them are the presence of certain alkaloids in plants, the existence of the human blood groups and the occurrence in relatively large amounts of the enzyme urease in soya and jack beans. However the number of such "ballast" mechanisms seems to be very small compared with the purposeful ones.

To sum up, the idea of purposiveness or wholeness is not only acceptable but essential to scientific thought. Its use however is to be a limited one. It is confined to biology and it differentiates in fact scientific thought in the non-biological subjects from that in biology. It must further be restricted to the formulation of questions and does not serve as an explanation.

In reply to von Brücke's remark quoted at the start, it might be said that, considering the variety of its form, teleology should be likened to a family rather than to a single individual. Some members of that family, some forms of teleology of Aristotle and his followers, are not respectable company for a scientist but others are. The biochemist who studies chemical substances or chemical reactions occurring in living matter finds it a help and stimulus when he asks what purpose a substance or reaction serves. In the field of energy production this viewpoint has led to a unified picture. The significances of many phenomena in this field can be appreciated if they are looked upon as part of a coherent whole. It is no less important that the viewpoint is a lead to new experiments.

II. COMMON FEATURES OF LIVING MATTER

I now wish to comment on another aspect of energy production in living matter. It is a striking fact that the basic features of the energy-producing mechanisms are identical, or very similar, in different types of organisms. ATP has been found in every living organism that has been searched with adequate techniques. The chief substrates of oxidative phosphorylation, the pyridine nucleotides, also occur universally. This suggests that the chief reaction responsible for the synthesis of ATP is universal. It has further been known for some time that the basic *anaerobic* mechanism of energy production by glycolysis is essentially the same in all animals and most micro-organisms. The tricarboxylic acid cycle is also known to occur in animals, plants and micro-organisms alike—although some micro-organisms possess, in all probability, alternative routes. As the substrate degradation is essentially a preparatory step to energy production proper, the micro-organisms, where the tricarboxylic acid cycle does not represent the main pathway, may still show the same energy-giving reactions proper.

Previously it had been realised that another aspect of the chemical make-up

of living matter, viz. its basic constituents, are also the same in all types of organisms. Essentially the same assembly of amino acids, the same sugars and the same fats are found throughout the living world.

The existence of common features in different types of living organisms is proof of a link between these organisms. A link is envisaged by evolutionary theory which assumes that the higher organisms, in the course of hundreds of millions of years, have arisen from the simpler ones. It would follow that the main energy-producing mechanisms must have been formed at a very early stage in the evolution of living matter.

It is true that information on energy production by micro-organisms is incomplete, and it is not impossible, though unlikely, that entirely different mechanisms exist in certain lower forms of life. It is extremely improbable that the same basic constituents, and the same energy-producing mechanisms should have arisen more than once and this leads to the conclusion that life in its present forms, has arisen only once.

III. "SIMPLICITY" OF LIVING MATTER

I have occasionally spoken of the "simplicity" of some features in the arrangement of the energy-producing mechanisms. To avoid misunderstandings I should emphasise that the "simplicity" refers to some basic principles which underlie the chemical organisation of living matter, but not to the final make-up. The latter is anything but simple, in fact unbelievably complicated. Considering that no two members even of the same species are identical the complexity of living organisms as a whole is obvious. This obvious variety and variability of living forms tends to obscure the fact that relatively simple principles may hide behind complex phenomena. Virtually infinite variety can be the result of the permutation and combination of a relatively small number of basic units. To bring this home I cannot do better than to quote a remark of an earlier Herter Lecturer, A. Kossel (13). The individuality of organisms is essentially an individuality of proteins and all proteins are but permutations and combinations of some twenty constituent amino acids all of which are derivatives of one parent substance, glycine. Kossel has remarked "We obtain some idea of the possible variety in the combinations and reactions of the protein constituents by recalling the fact that they are as numerous as the letters of the alphabet which are capable of expressing an infinite number of thoughts."

It has been said that the search for simple principles may lead to fallacies. Whitehead (14) made the remark "Seek simplicity and distrust it." I do not think that we need distrust the kind of simplicity which we have encountered in the chemical make-up of living matter; it is in accord with the evolutionary concepts and is therefore not unexpected.

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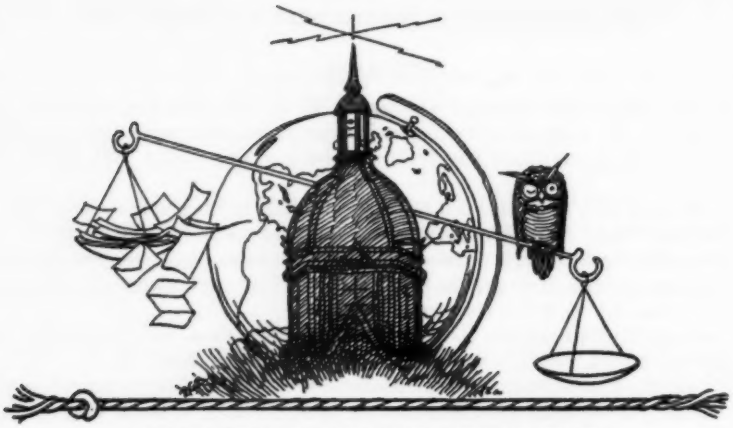

BOOK REVIEWS

(These reviews represent the individual opinions of the reviewers and not necessarily those of members of the Editorial Board of this Bulletin)

Physics for Medical Students. By J. S. ROGERS. 405 pp., $5.50. Cambridge University Press, New York.

This is an unusual physics text-book and its title gives little indication of its character. The author in his preface states that his main aim has been to supplement standard texts of physics in order to provide special information of interest and value to medical students and to medical graduates with research interests involving physical principles. On this basis therefore, it must be judged; the lack of any exposition of basic physics certainly renders it useless as a single text for any student.

Although the book is small a great deal of information is packed into it. The chapters can be divided into three groups. Of these the largest group is concerned with matters of pure physics. Here the book is at its best and is generally up to date including, for instance, detailed descriptions of atomic transformations and of the structure and function of various types of atomic piles. It is doubtful, however, to what extent the larger part of this information, though of great interest, is useful to the average student of medicine. The medical graduate whose research might lead him to require knowledge in these fields would probably prefer to consult an appropriate work.

The author acknowledges assistance from several authorities in preparing the chapters which constitute the above group, but no mention is made of assistance with those which form the next group, devoted to physical chemistry and biophysical considerations. This lack of assistance may account for the weakness of this part of the book. These chapters contain little that cannot be found expounded better in text-books of physical chemistry, anatomy and physiology. They are marred by several inaccuracies. The chapter on the circulation is notably very poor. In contrast to the up-to-date information listed above there is no mention of many developments which are far from new. In the chapter on electrocardiography, which is very disappointing, the author appears to be only just aware that an apical precordial lead is being used. No mention is made of the other chest leads or of the important physical aspects of the transmission of electrical signals through volume conductors, although this last would seem an ideal topic for a book of this type.

The last group consists of chapters devoted to the history of physics. These are well done and interesting but seem to have no particular relevance to the object of the book.

The standard of writing and illustration is mixed; in some parts the exposition is very lucid and starts on an elementary level, whereas in many others it would be difficult to follow the author without already possessing a considerable knowledge of the subject under discussion. The figures are mostly very simple line drawings. Some benefit by this simplicity but too often it results in an inadequate, sometimes inaccurate, picture which provides little assistance in following the text. The paper, printing, and binding are all good.

Most students, and probably many medical graduates, would enjoy glancing through this book and would find many interesting pieces of information by so doing, but it cannot be recommended that they should purchase it or plan to use it as a regular text from which to study.

D. C. D.

You and Your Health. By EDWIN P. JORDAN. 296 pp., $3.95. G. P. Putnam's Sons. New York.

The author, Dr. Edwin P. Jordan, a graduate of Rush Medical College in Chicago in 1928, taught at both Chicago and Northwestern, and now is lecturer in Social and Environmental Medicine at the University of Virginia. He is also the author of a syndicated newspaper column entitled "The Doctor Says".

You and Your Health is written in simple language for the lay reader and enables him to pick up a great deal of useful and accurate information in using home remedies. The author discusses minor ailments extensively and "lays the ghost" of some ancient beliefs such as the production of warts from handling toads.

The importance of this book, however, lies in its attitude towards serious diseases. Editors of the popular magazines do not hesitate to dramatize the painful and fatal aspect of disease in order to sell more copy. Even the well adjusted individual can be fearful of the unknown, and by reading this lurid medical literature can become preoccupied with some minor pains and develop a neurosis. Every practitioner, internist, and surgeon knows how apprehensive many of their patients are and how sensitive they are to threats to their health. These doctors know that the practice of medicine is becoming increasingly hard because of these implied threats. Names of diseases such as arteriosclerosis, cancer, and multiple sclerosis are becoming known to everyone. Diagnosis is becoming increasingly difficult because of the distortion of the history by pseudo-knowledge which the patient has gained from this reading.

Dr. Jordan takes up many serious and fatal diseases and discusses them with a calm objectivity that is very reassuring. The frequent escape from apparently hopeless situations is very helpful. Where there are uncertain variables, Dr. Jordan is usually bullish on the outcome. Many important diseases have been left out, but Dr. Jordan is thinking of writing another book. The vascular system, cancer, and injections are particularly well discussed.

It is a book that in large measures counterbalances the impact of popular dramatic medical literature and might be very useful to many an apprehensive patient.

RALPH G. HILL

Anatomy for Surgeons, Vol. 1. The Head and Neck. By W. HENRY HOLLINSHEAD. 560 pp., $10.00. Paul B. Hoeber, New York.

This is the first of a three volume set which apparently will cover the entire field of anatomy. The present volume is concerned with the head and neck and contains sections devoted to the cranium, the orbit, the ear, the nose and paranasal sinuses, the fascia and fascial spaces of the head and neck, the face, the jaws, palate and tongue, the pharynx and larynx, and the neck.

The regions considered in this volume are well covered. The material is logically organized, and information on a specific subject or region is easily found. In general, all anatomical aspects of a given region are considered together, making it very satisfactory for reference purposes. The text is directed consistently toward the surgically important aspects of anatomy and frequently toward specific surgical problems, but without an attempt to include operative surgery. The emphasis always remains clearly on anatomy. This orientation is distinctive and is to be praised. Perhaps one of the outstanding features of the book is the admirably successful attempt which the author has made to correlate the anatomical features with the pertinent embryology and surgical physiology of each region. Such perspective is essential in a thoughtful consideration of surgical problems. The text is well written and easy to follow.

The discussions are well illustrated by an adequate number of diagrams. Since many excellent anatomical atlases are already available, little could be gained by the publication of another, and since, in the many instances, the points with which this book is concerned can be more satisfactorily illuminated by such diagrams than by exact anatomical drawings, it would seem that this represents a wise choice. The bibliography is extensive, and contains many references to recent pertinent work. The book is printed in easily readable type on nonreflecting paper.

It would seem to the reviewer that the addition of this book to the already extensive library of surgical anatomical texts is adequately justified by the unusually consistent orientation of approach. While directed toward clinical problems, it is the pertinent anatomy, embryology and physiology which are stressed, rather than clinical or technical elements. The emphasis on a well organized, coherent, descriptive text rather than on extensive illustions is different and highly satisfactory. The book is well organized for use both during the process of learning the surgical skills and as a reference text at a later date. It is the reviewer's opinion that a text such as this might well find a place in the instruction of basic anatomy, where the most important purpose of the text and instructor must be to stimulate the student's interest. This can be accomplished perhaps best by correlation of pure anatomic knowledge with clinical application, yet with emphasis always on the former. This book is well-suited to such an approach.

If the remaining volumes are similarly organized and written, this group of books should make a real addition to the anatomical library of the student and surgeon.

JAMES R. CANTRELL

Skin Therapeutics. Prescription and Preparation. (Materia Medica Dermatologica). By M. K. POLANO with an Introduction by CLARENCE S. LIVINGOOD. 276 pp., $6.00. Elsevier Press, Houston, Texas.

The author is well known for extensive experimental work on topical therapeutics. He points out that: "in spite of all our wishful thinking about the internal management of skin diseases, external therapeutics is still our most powerful weapon against most dermatoses—there is indeed a vast and scattered knowledge about a great number of therapeutic agents and the vehicles apt to convey them to the skin, but a really sound scientifically based foundation is often lacking."

The purpose of his book is threefold: (1) to present the results of his numerous experiments and experience, (2) to stress the importance of the art of prescription writing which is seriously threatened by the modern trend of prescribing proprietary drugs, and (3) to present to an international forum a summary of important and useful drugs from the United States Pharmacopoeia, the British Pharmacopoeia, the Pharmacopoeia Helvetica and the Pharmacopoeia Neerlandica, in order to stimulate understanding on common ground.

The material is well presented and in the opinion of the reviewer it is the most comprehensive and authoritative work of its kind.

MAURICE SULLIVAN

Cancer Cytology of the Uterus. By J. ERNEST AYRE. 407 pp., $14.50. Grune & Stratton, Inc., New York.

Cancer Cytology of the Uterus by J. Ernest Ayre is timely, authoritative and complete. Cytological diagnosis after more than ten years of exhaustive clinical application has passed beyond the trial stage and is now well established as a valid and practical means of indicating the presence or absence of malignancy. Today the cytological smear is an integral part of the gynecological examination and the gynecologist has little choice but to utilize it.

The author by his numerous excellent contributions to the field of cytology and his constant and enthusiastic devotion to its merits is eminently qualified to present this monograph in this chosen field. The subject matter is well organized, presented in a simple, orderly fashion and magnificently illustrated. It quite adequately covers "cytological diagnosis within the limitations of the gynecological field".

In 396 pages, including 360 photomicrographs, the author conducts us through the development of the cytological concept, the technique of obtaining and preparing the smear and the identification of the normal, abnormal and malignant cells to be found exfoliated in the human vagina. Unlike previous texts which have concentrated upon illustrating the cell findings in frank carcinoma, sufficient attention has been paid to the extensive range of cell manifestations found in non-malignant disease and familiarity with which is of prime importance in the education of the cytologist.

Chapter Five, utilizing almost one hundred pages, Dr. Ayre devotes to his own personal contribution "the pre-cancer cell complex". The author describes this as a stage closely related to the "in situ" stage although characterized by cell types believed to be of an incompletely developed malignant type. This particular phase of the subject is at present still controversial and one may accuse the author of being a bit presumptious in his discussion of it. One must admit that Dr. Ayre points out how the cytological method possesses great potentialities; not only with regard to its practical usefulness in cancer diagnosis but also in its more fundamental value in cancer research.

Perhaps the most striking and pleasant aspect of this monograph is the manner in which Dr. Ayre documents his cytological interpretations with accompanying histology as found in biopsies or the removed uterus. This feature not only adds interest to his presentation but makes cytological diagnosis more convincing and realistic to the novice.

To the cytologist or the prospective cytologist this monograph will prove not only a welcome and authoritative treatise but almost a "vade mecum". To the gynecologist with only a practical interest in cytology this book should be a striking addition to his library. It will be useful only if one is content to spend countless hours at the microscope along with it. To Dr. Ayre it should be a source of justified pride.

GERALD A. GALVIN

Streptococcal Infections. New York Academy of Medicine Section on Microbiology Symposium Number Seven. Edited by MACLYN MCCARTY. 218 pp., $5.00. Columbia University Press, New York.

Although the recent advent of several chemotherapeutic agents has brought about significant reduction of the mortality and morbidity resulting directly from infections with beta hemolytic streptococci, a number of problems concerning the evolution of such disorders and their probable sequellae remain unsolved. In the present volume, results of continuing

studies on the biology of streptococci and infections caused by these organisms are presented and integrated with knowledge previously extant by acknowledged authorities in the field. The first five chapters are devoted to bacteriologic investigations, the remaining ten to the several aspects of host-parasite relationships including epidemiology, immunology, sequellae of infection and the effects of chemoprophylaxis upon the carrier state and the incidence of rheumatic fever. The material is set forth in lucid style by fifteen of the sixteen participants in the Seventh Symposium of the Section on Microbiology of the New York Academy of Medicine and provides a ready source of information on a number of developing concepts in this active field. The usefulness of the work is heightened further by the inclusion of extensive bibliographies.

ROBERT AUSTRIAN

BOOKS RECEIVED FOR REVIEW

Atlas of Operative Technic Anus, Rectum and Colon. By HARRY E. BACON AND STUART T. ROSS. 301 pp., $13.50. The C. V. Mosby Co., St. Louis, Missouri.

Atlas of Orthopedic Traction Procedures. By CARLO SCUDERI. 230 pp., $12.50. The C. V. Mosby Co., St. Louis, Missouri.

The Effect of ACTH and Cortisone upon Infection and Resistance. New York Academy of Medicine, Section on Microbiology, Symposium Number Six. Edited by GREGORY SHWARTZMAN. 204 pp., $5.50. Columbia University Press, New York.

Electrocardiography. By E. GREY DIMOND. 261 pp., $14.00. The C. V. Mosby Co., St. Louis, Missouri.

Geriatric Medicine, 3rd edition. By EDWARD J. STIEGLITZ. 718 pp., $15.00. J. B. Lippincott Co., Philadelphia, Pa.

The Improvement of Patient Care. A Study at Harper Hospital. By MARION J. WRIGHT, Assoc. Dir. Harper Hospital, Detroit, Mich. 236 pp. G. P. Putnam's Sons, New York, N. Y.

Toxicity of Industrial Organic Solvents. By E. BROWNING. 411 pp., $8.00. Chemical Publishing Co., New York, N. Y.

Wine as Food and Medicine. By SALVATORE P. LUCIA. 149 pp., $3.00. The Blakiston Co., New York, N. Y.